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(54) **NOVEL TUBULYSIN ANALOGUES**

(75) Inventors: **Alexander Domling**, Munchen (DE); **Bernd Henkel**, Planegg (DE); **Barbara Beck**, Bethesda, MD (US); **Katrin Illgen**, Planegg (DE); **Sukumar Sakamuri**, San Diego, CA (US); **Sanjay Menon**, Plainsboro, NJ (US)

Correspondence Address:
EDWARDS & ANGELL, LLP
P.O. BOX 55874
BOSTON, MA 02205 (US)

(73) Assignee: **R&D-Biopharmaceuticals Am**, Martinsried (DE)

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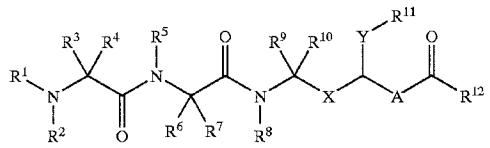
(57) **ABSTRACT**

(II)

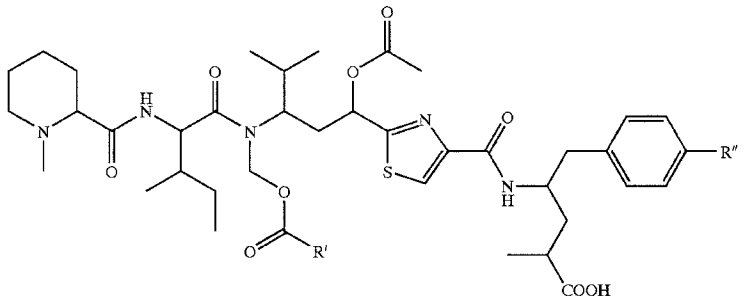


The invention relates to tubulysin derivatives of general formula (II), said derivatives having a cytostatic effect.

NOVEL TUBULYSIN ANALOGUES

[0001] The present invention refers to novel tubulysin analogs and its use for the treatment of cancer diseases.

[0002] Tubulysins, for the first time were isolated by Höfle and Reichenbach et al. (GBF Braunschweig) from a culture browth of the myxobacterial strains of *Archangium gephyra* (F. Sasse et al. J. Antibiot. 2000, 53, 879-885; WO9813375; DE 10008089). These compounds show high cytotoxicity in the low picomolare IC_{50} in a panel of cancer cell lines; thus they are of interest as potential anticancer therapeutics. Tubulysins (I) are tetrapeptides, containing three unusual amino acids; thus the total synthesis pose a considerable challenge to organic chemists.



(I)

[0003] Tubulysin A: R'=$CH_2CH(CH_3)_2$; R"=OH

[0004] Tubulysin B: R'=$CH_2CH_2CH_3$; R"=OH

[0005] Tubulysin C: R'=CH_2CH_3; R"=OH

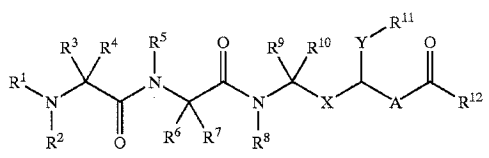
[0006] Tubulysin D: R'=$CH_2CH(CH_3)_2$; R"=H

[0007] Tubulysin E: R'=$CH_2CH_2CH_3$; R"=H

[0008] Tubulysin F: R'=CH_2CH_3; R"=H

[0009] It is an objective of the present invention to provide novel Tubulysin analogues with improved activity and properties, in particular pharmacological properties as compared to the natural products.

[0010] The present invention provides a compound of Formula (II):



(II)

[0011] wherein

[0012] A is a substituted 5- or 6-membered heteroaryl;

[0013] wherein

[0014] X is O, S or NR^{13} or $CR^{14}R^{15}$;

[0015] wherein

[0016] Y is O, S or NR^{16};

[0017] R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16} are independently H, alkyl, alkenyl, alkinyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, heterocycloalkyl, aralkyl or heteroaralkyl, or two R's are members of a cycloalkyl or heterocycloalkyl ring system;

[0018] wherein compounds of Formula (I) are excluded,

[0019] wherein R' are H, alkyl, alkenyl, aryl or heteroaryl and—at the same time—R" are H, —OH, alkyl, aryl, or heteroaryl;

[0020] or a pharmacologically acceptable salt, a solvate, a hydrate or a pharmacologically acceptable formulation thereof. Explicitely excluded are Tubulysins A, B, C, D, E and F.

[0021] The term alkyl or alk refers to a saturated, linear or branched hydrocarbon group, containing from one to twenty carbon atoms, preferably from one to twelve carbon atoms, mostly preferred from one to six carbon atoms, for example methyl, ethyl, propyl, isoporpyl, isobutyl, n-butyl, tert-butyl, n-hexyl, 2,2-dimethylbutyl or n-octyl.

[0022] The term alkenyl and alkinyl refers to a at least partially unsaturated, linear or branched hydrocarbon group, containing from two to twenty carbon atoms, preferably from two to twelve carbon atoms, mostly preferred from two to six carbon atoms, for example ethenyl, allyl, acetylenyl, prpargyl, isoprenyl, or hex-2-enyl. Preferentially alkenyl groups contain one or two, mostly preferred one double bond and alkinyl group contain one or two, mostly preferred one triple bond.

[0023] Optionally the term akyl, alkenyl and alkinyl refers to groups where one or several, preferentially one, two or three hydrogen atoms are replaced by a halogen atom, prferentially fluorine or chlorine or a 2,2,2-trichlorethyl, or a trifluoromethyl.

[0024] The term heteroalkyl refers to a alkyl, alkenyl or alkinyl group, where several, preferentially one, two or three carbon atoms are replaced by a O, N, P, B, Se, Si, or S atom,

preferentially O, S, N. The term heteroalkyl refers to a carboxylic acid or a thereof derived group, for example acyl (alkyl-CO), acylalkyl, alkoxycarbonyl, acyloxy, acyloxyalkyl, carboxyalkylamid or alkoxycarbonyloxy.

[0025] Examples of heteroalkyl groups are groups of the formula R^a—O—Y^a—, R^a—S—Y^a, R^a—N(R^b)—Y^a—, R^a—CO—Y^a—, R^a—O—CO—Y^a—, R^a—CO—O—Y^a—, R^a—CO—N(R^b)—Y^a—, R^a—N(R^b)—CO—Y^a—, R^a—O—CO—N(R^b)—Y^a—, R^a—N(R^b)—CO—O—Y^a—, R^a—N(R^b)—CO—N(R^c)—Y^a—, R^a—O—CO—O-ZY^a—, R^a—N(R^b)—C(=NR^d)-N(R^c)—Y^a, R^a—CS—Y^a—, R^a—O—CS—Y^a—, R^a—CS—O—Y^a, R^a—CS—N(R^b)—Y^a—, R^a—N(R^b)—CS—Y^a—, R^a—O—CS—N(R^b)—Y^a—, R^a—N(R^b)—CS—O—Y^a—, R^a—N(R^b)—CS—N(R^c)—Y^a—, R^a—S—CO—Y^a—, R^a—CO—S—Y^a—, R^a—S—CO—N(R^b)—Y^a—, R^a—N(R^b)—CO—S—Y^a—, R^a—S—CO—O—Y^a—, R^a—CO—S—Y^a—, R^a—S—CO—S—Y^a—, R^a—S—CS—Y^a—, R^a—CS—S—Y^a—, R^a—S—CS—N(R^b)—Y^a—, C_1-C_6-alkyl, a C_2-C_6-alkenyl or a C_2-C_6-alkinyl group; wherein R^b refers to a H, a C_1-C_6-alkyl, a C_2-C_6-alkenyl or a C_2-C_6-alkinyl group; wherein R^c refers to a H, a C_1-C_6-alkyl, a C_2-C_6-alkenyl or a C_2-C_6-alkinyl group; wherein R^d refers to a H, a C_1-C_6-alkyl, a C_2-C_6-alkenyl or a C_2-C_6-alkinyl group and Y^a refers to a direct binding, a C_1-C_6-alkylen, a C_2-C_6-alkenylen or a C_2-C_6-alkinylen group, wherein each heteroalkyl group can be replace by a carbon atom and one or several hydrogen atoms can be replaced by fluorine or chlorine atoms. Examples of heteroalkyl groups are methoxy, trifluormethoxy, ethoxy, n-propyloxy, iso-propyloxy, tert-butyloxy, methoxymethyl, ethoxymethyl, methoxyethyl, methylamino, ethylamino, dimethylamino, diethylamino, isopropylethylamino, methyl-aminomethyl, ethylaminomethyl, di-iso-propylaminoethyl, enolether, dimethylaminomethyl, dimethylaminoethyl, acetyl, propionyl, butyryloxy, acetyloxy, methoxycarbonyl, ethoxy-carbonyl, N-ethyl-N-methylcarbamoyl or N-methylcarbamoyl. Other examples of heteroalkyl groups are nitrile, isonitrile, cyanate, thiocyanate, isocyanate, isothiocyanate and alkylnitrile groups.

[0026] The term cycloalkyl refers to a saturated or partially unsaturated (e.g. cycloalkenyl) cyclic group, comprising one or several rings, preferntialy one or two, containing three to fourteen ring carbon atoms, preferentially three to ten, preferentially three, four, five, six or seven ring carbon atoms. Furthermore the term cycloalkyl refers to a group where one or more hydrogen atoms are replaced by F, Cl, Br, I, OH, =O, SH, =S, NH_2, =NH, or NO_2, or cyclic ketones, for example cyclohexanone, 2-cyclohexenone or cyclopentanone. Examples of cycloalkyl groups are cyclopropyl, cyclobutyl, cyclopentenyl, spiro[4,5]-decanyl, norbornyl, cyclohexyl, cyclopentenyl, cyclohexadienyl, decalinyl, cubanyl, bicyclo[4.3.0]nonyl, tetralin, cyclopentylcyclohexyl, fluor-cyclohexyl or the cyclohex-2-enyl group.

[0027] The term heterocycloalkyl refers to the above definition, wherein a or several, preferentialy one, two or three ring carbon atoms are replaced by a O, N, Si, Se, P, or S, prferentially O, S, N. Preferentially a heterocycloalkyl goups is composed of one or two rings comprising three to ten, preferentially three, four, five, six or seven ring atoms. Moreover the term heterocycloalkyl refers to groups where a or several hydrogen atoms are replaced by F, Cl, Br, I, OH, =O, SH, =S, NH_2, NO_2. Examples of heterocycloalkyl are piperidyl, morpholinyl, urotropinyl, pyrrolidinyl, tetrahydrothiophenyl, tetrahydropyranyl, tetrahydro-furyl, oxacyclopropyl, azacyclopropyl or 2-pyrazolinyl groups as well as lactams, lactons, cyclic imides and cyclic anhydrides.

[0028] The term alkylcycloalkyl refers to groups, which contain cycloalkyl as well as alkyl, alkenyl or alkinyl groups according to the above definition, e.g. alkylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkyl and alkinylcycloalkyl groups. Preferentially a alkylcycloalkyl group is composed of a cycloalkyl group, comprising one or more rings, comprising three to ten, preferentially three, four, five, six or seven carbon—atomes and one or two alkyl, alkenyl oder alkinyl groups with one or two to six carbon atoms.

[0029] The term heteroalkylcycloalkyl refers to alkylcycloalkyl groups, according to the above definition, wherein one or several, preferentialy one, two or three carbon atoms are replaced by O, N, Si, Se, P or S, preferentialy O, S, N. Preferentially it is composed of a heteroakylcycloalkyl group comprising one or two ring systems with three to ten, preeferentialy three, four, five, six or seven ring atoms and one or two alkyl, alkenyl, alkinyl or heteroalkyl groups with one or two to six carbon atoms. Examples of such a group are alkylheterocycloalkyl, alkylheterocycloalkenyl, alkenylheterocycloalkyl, alkinylheterocycloalkyl, heteroalkyl-cycloalkyl, heteroalkylheterocycloalkyl and heteroalkylheterocylcloalkenyl, wherein the cyclic group is saturated or partially (simply, twofold or threefold) unsaturated.

[0030] The term aryl or ar refers to a aromatic group, composed of one or several rings, comprising six to fourteen carbon atoms, preferentially six to ten, prferentially six carbon atoms. The term aryl or ar refers to a aromatic group, wherein one or several H atoms are replaced by F, Cl, Br or I or OH, SH, NH_2, or NO_2. Examples are phenyl-, naphthyl-, piphenyl-, 2-fluorphenyl, anilinyl-, 3-nitrophenyl or 4-hydroxy-phenyl.

[0031] The term heteroaryl refers to a aromatic group, composed of one or several rings, comprising five to fourteen rind atoms, preferentially five to ten, and a or several, preferentially one, two, three or four O, N, P or S ring atoms, prferentially O, S or N. The term heteroaryl refers to groups, wherein one or several H atoms are replaced by F, Cl, Br or I or OH, SH, NH_2, or NO_2. Examples are 4-pyridyl, 2-imidazolyl, 3-phenylpyrrolyl, thiazolyl, oxazolyl, triazolyl, tetrazolyl, isoxazolyl, indazolyl, indolyl, benzimidazolyl, pyridazinyl, chinolinyl, purinyl, carbazolyl, acridinyl, pyrimidyl, 2,3'-bifuryl, 3-pyrazolyl and iso-chinolinyl.

[0032] The term aralkyl refers to groups, in accordance to the above definition, composed of aryl and alkyl, alkenyl, alkinyl and/or cycloalkyl, e.g. arylalkyl, arylalkenyl, arylalkinyl, arylcycloalkyl, arylcycloalkenyl, alkylarylacycloalkyl and alkylarylcycloalkenyl. Examples of aralkyles are toluol, xylol, mesitylen, styren, benzylchloride, o-fluortoluene, 1H-inden, tetralin, dihydronaphthaline, indanon, phenylcyclopentyl, cumol, cyclo-hexylphenyl, fluoren and indan. Preferentially, a aralkyl group is composed of composed of one or two aromatic rings, comprising six to ten ring carbon atoms and one or two alkyl, alkenyl and/or alkinyl comprising one or two to six carbon atoms and/or one cyclo-alkyl comprising five or six ring carbon atoms.

[0033] The term heteroaralkyl refers to groups, in accordance to the above definition, wherein one or several, preferentially one, two, three or four carbon atoms are

replaced by O, N, Si, Se, P, B, S, preferentially O, N or S, and groups which according to the above definition contain aryl, heteroaryl and alkyl, alkenyl, alkinyl and/or heteroalkyl and/or cycloalkyl cnd/or heterocyclo-alkyl. Preferentially a heteroaralkyl group is composed od a or two aromatic ring systemes comprising five or six to ten carbon atoms and one or two alkyl, alkenyl and/or alkinyl comprising one or two to six carbon atoms and/or one cycloalkyl comprising five or six ring carbon atoms, wherein one, two, three or four carbon atoms can be replaced by O, N or S.

[0034] Examples are arylheteroalkyl, arylheterocycloalkyl, arylheterocycloalkenyl, arylalkylheterocycloalkyl, arylalkenylheterocycloalkyl, arylalkinylheterocyclo-alkyl, arylalkylheterocycloalkenyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkinyl, heteroarylheteroalkyl, heteroarylcycloalkyl, heteroarylcycloalkenyl, heteroarylheterocycloalkyl, heteroarylheterocycloalken-yl, heteroarylalkylcycloalkyl, heteroarylalkylhetero-cycloalkenyl, heteroarylheteroalkylcycloalkyl, Hetero-arylheteroalkylcycloalkenyl and heteroarylheteroalkyl heterocycloalkyl, wherin the cyclic groups can be saturated or simple, twice, three fold of four fold unsaturated. Examples are tetrahydroisochinolinyl, benzoyl, 2- or 3-ethyl-indolyl, 4-methylpyridino, 2-, 3- or 4-methoxyphenyl, 4-ethoxyphenyl, 2-, 3- or 4-carboxyphenylalkyl.

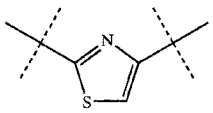
[0035] The terms cycloalkyl, heterocycloalkyl, alkylcyclo-alkyl, heteroalkylcycloalkyl, aryl, heteroaryl, aralkyl and heteroaralkyl refer to groups, wherein one or several H atoms are replaced by F, Cl, Br or I or OH, SH, NH_2, or NO_2.

[0036] The term "optinally substituiert" relates to groups, wherein one or several H atoms are replaced by F, Cl, Br or I or OH, SH, NH_2, or NO_2. The term "gegebenenfalls substituiert" relates further to groups, comprising exclusively or in addition unsubstituted C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkinyl, C_1-C_6 heteroalkyl, C_3-C_{10} cycloalkyl, C_2-C_9 heterocycloalkyl, C_6-C_{10} aryl, C_1-C_9 heteroaryl, C_7-C_{12} aralkyl or C_2-C_{11} heteroaralkyl.

[0037] Protecting groups are known to the specialist and described in P. J. Kocienski, Protecting Groups, Georg Thieme Verlag, Stuttgart, 1994 and in T. W. Greene, P. G. M. Wuts, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, 1999. Common amino protecting groups are e.g. t-butyloxycarbonyl (Boc), benzyloxycarbonyl (Cbz, Z), benzyl (Bn), benzoyl (Bz), fluorenylmethyloxycarbonyl (Fmoc), allyloxycarbonyl (Alloc), trichlorethyloxycarbonyl (Troc), acetyl or trifluoracetyl.

[0038] Compounds of Formula (II) can comprise several chiral centers related to their substitution pattern. The present invention relates to all al defined enantio and diastereo isomers as well as their mixtures in all ratios. Moreover the present invention relates to all cis/trans isomers of compounds of the general Formula (II) as well as thier mixtures. Moreover the present invention relates to all tautomeric forms of compounds of the general Formula (II).

[0039] Preferably A constitutes a optinally substituted thizol ring; more preferably A has the following structure:



[0040] Moreover preferably X constitutes a CH_2 group.

[0041] Preferably Y constutites O.

[0042] Preferably R^1 constitutes a C_1-C_4 alkyl.

[0043] Preferably R^2 ans R^3 constitute together $(CH_2)_n$ with n=2, 3, 4 or 5.

[0044] Preferably R^4 constitutes H or methyl.

[0045] Preferably R^5 constitutes H.

[0046] Preferably R^5 constitutes C_1-C_6 alkyl, C_3-C_6 cycloalkyl or C_4-C_7 lkylcycloalkyl.

[0047] Preferably R^5 constitutes H or methyl.

[0048] Preferably R^8 constitutes CH_2OCOR^{17}, wherein R^{17} constitutes C_1-C_6 alkyl or C_1-C_6 alkenyl.

[0049] Preferably R^9 constitutes C_1-C_6 alkyl.

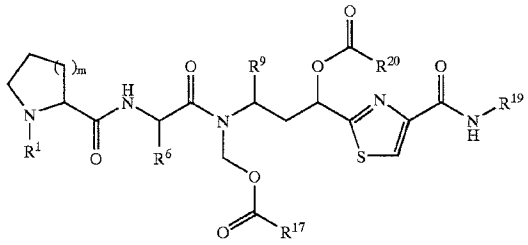
[0050] Preferably R^{10} constitutes H or methyl.

[0051] Preferably R^{11} constitutes H or —(C═O)—(C_{1-4})Alkyl.

[0052] Preferably R^{12} constitutes $NR^{18}R^{19}$, wherein R^{18} constitutes H or methyl and R^{19} constitutes aralkyl or heteroaralkyl.

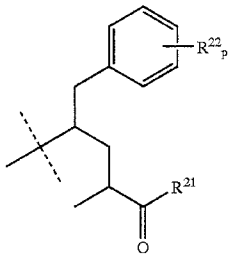
[0053] Most preferably are compounds of Formula (III),

(III)



[0054] wherein R^1 comprise C_1-C_4 alkyl, R^6 comprise C_1-C_6 alkyl, R^9 comprise C_1-C_6 alkyl, R^{17} comprise C_1-C_6 alkyl or C_1-C_6 alkenyl, R^{19} comprise aralkyl or heteroaralkyl, R^{20} comprise C_1-C_4 alkyl and m equals 1 or 2.

[0055] Preferentially R^{19} comprise the following structure:



[0056] wherein R^{21} comprise OH, NH_2, alkyloxy, alkyl amino or dialkyl amino, R^{22} comprise halogen, OH, NO_2, NH_1, alkyloxy, alkyl amino or dialkyl amino and p equals 0, 1, 2 or 3.

[0057] Examples of pharmacologically acceptable salts of compounds of Formula (II) are physiologically acceptable mineral acids, e.g. hydrochloric acid, sulfuric acid, phor-

phoric acid or salts of organic acids, e.g. methansulfonic acid, p-toluenesulfonic acid, lactic acid, formic acid, trifluoracetic acid, citric acid, succinic acid, fumaric acid, maleic acid and salicylic acid. Compounds of Formula (II) can be solvated, especially hydrated. The hydration can occur during the synthesis process or can be a consequence of the hygroscopic nature of the originally dehydrated compound of Formula (II). Compounds of Formula (II), containing assymetric carbon atoms might exist as mixtures of diastereomers, as mixtures of enantiomers or as optically pure compounds.

[0058] The pharmaceutical composition according to the present invention is composed of at least one compound of Formuly (II) and optinally carrier and/or adjuvants.

[0059] Prodrugs are also subject of the present invention and they are composed of a compound of Formula (II) and at least one pharmakologically acceptable protecting group, which is cleaved under physilogical conditions, e.g. alkoxy, aralkyloxy, acyl or acyloxy, more precicely ethoxy, benzyloxy, acetyl or acetyloxy. Moreover the present invention relates to conjugates comprising at least one compound of Formula (II) and a biological macromolecule, e.g. oligo saccharide, monoclonale antibody, lectine, PSA (prostata specific antigen) or peptidic vectors and if needed as well as a suitable linker. The expression linker relates to a chemical group, which links compounds of Formula (II) with a biological macromolecule. Examples of linkers are alkyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, heterocycloalkyl, aralkyl or heteroaralkyl.

[0060] The therapeutic usage of compounds of Formula (II), its pharmacologic acceptable salts and/or its solvates and hydrates, as well as the corresponding formulations and pharmacological compositions are also subject of the present invention.

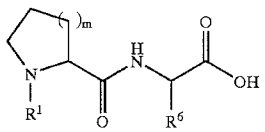
[0061] The usage of the active agents for the preperation of drugs for the treatment of cancer is also subject of the present invention. Moreover the present compounds are of interest for the prevention and/or treatment of rheumatoid arthritis, inflammatory diseases, immunological diseases (e.g. type I diabetis), autoimmune diseases, other tumor diseases as well as for the surface treatment (impregnation) of plastic and metal implants, e.g. stents. In general, compounds of Formula (II) will be given as a single treatment or in combination with an arbitrary therapeutic substance according to known and accepted modes. Such therapeutically useful compositions can be administered in one of the following ways: orally, including dragees, coated tablets, pills, semi-solids, soft or hard capsules, solutions, emulsions or suspensions; parenteral, including injectable solutions; rectal as suppositories; by inhalation, including powder formulation or as a spray, transdermal or intranasal. For the production of such tablets, pills, semi solids, coated tabletts, dragees and hard gelatine capsules the therapeutically used product is mixed with pharmacologically inert, anorganic or organic carriers, e.g. with lactose, sucrose, glucose, gelatine, malt, silical gel, starch, or derivatives thereof, talkum, stearinic acid or its salts, dried skim milk and the like.

[0062] For the production of soft capsuls a carrier one may use for example vegetable oils, petroleum, animal or synthetic oils, wax, fat, polyols. For the production of liquide solutions and syrups one may use carriers for example water, alcohols, aqueous saline, aqueous dextrose, polyole, glycerin, vegatable oils, petroleum, animal or synthetic oils. For the production of suppositories one may use excipients as are e.g. vegetable, petroleum, animal or synthetic oils, wax, fat and polyols. For aerosol formulations one may use compressed gases suitable fort his purpose, as are e.g. oxigen, nitrogen, noble gas and carbon dioxide. The pharmaceutically useful agents may also contain additives for conservation, stabilisation, e.g. UV stabilizer, emulsifier, sweetener, aromatiser, salts to change the osmotic pressure, buffers, coating additives and antioxidants.

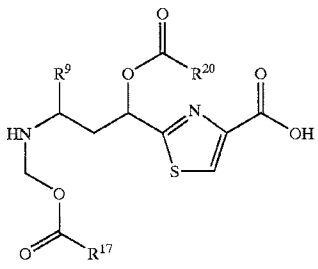
[0063] Combinations with other therapeutic agents can include further agents, which are commonly used to treat cancer.

[0064] Compounds of Formula (IV), (V) and (VI) provided with suitable protecting groups are produced as building blocks for the of compounds of Formula (II). These can be linked via peptide coupling methods using known coupling reagents, e.g. hydroxybenzotriazole (HOBT) and diisopropylcarbodiimide (DIC) or dicyclohexylcarbodiimide (DCC).

(IV)



(V)

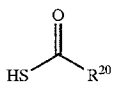


(VI)

[0065] Building block (IV) can be assembled through peptide coupling of commercially availbable and known aminoacids.

[0066] Building block (V) can be assembled through a muticomponent reaction of starting materials of Formula (VII), (VIII) und (IX).

(VII)



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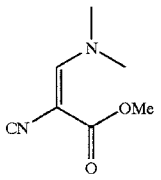

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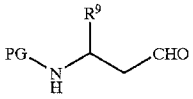
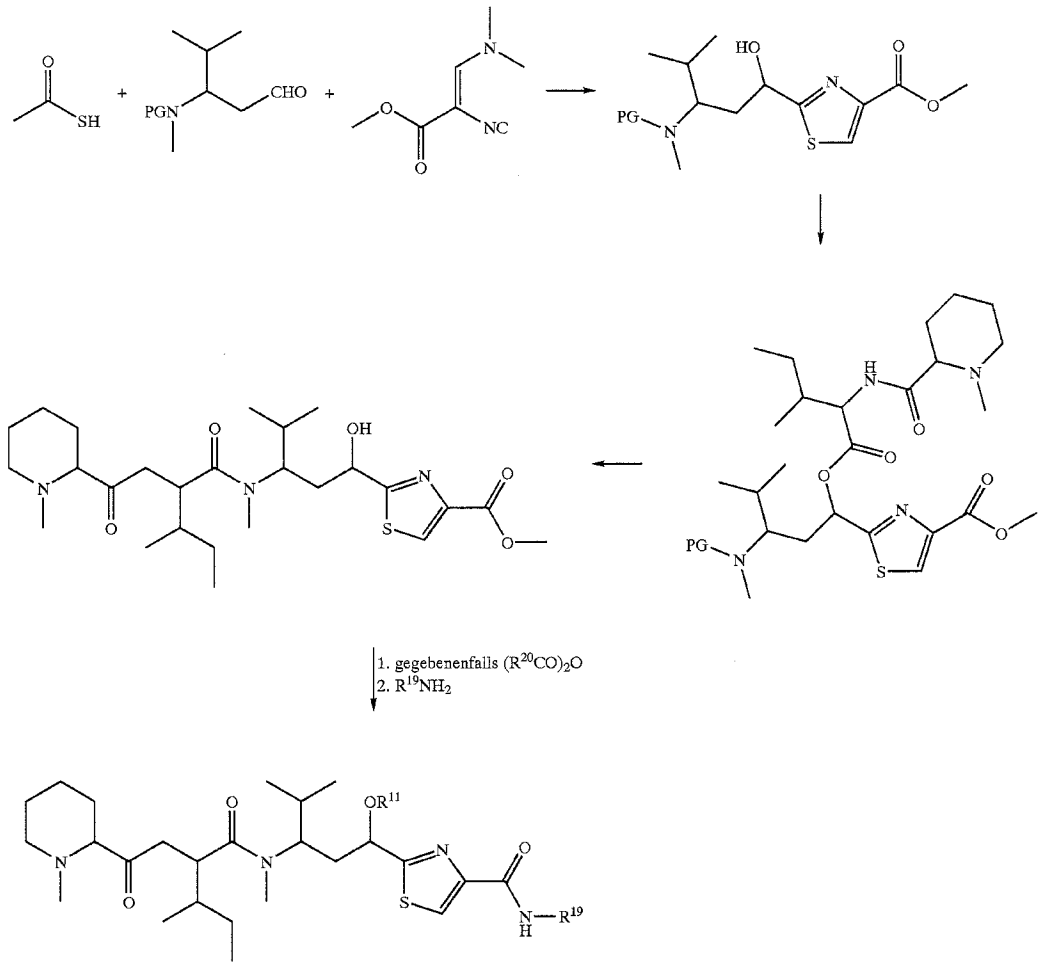
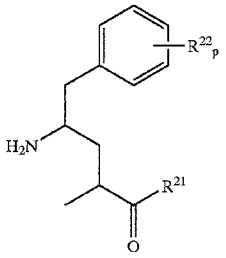


[0067] Herein PG is a known amino protecting group, for example tert-butyloxycarbonyl (Boc). The resulting compound can be further transformed to building block (V) using $R^{17}COOCH_2Cl$ or H_2CO and $R^{17}COOH$ or H_2CO, TMS-Cl and $R^{17}COONa$ (I. Kornonen et al. Acta Chem. Scand. Ser. B 1982, 36(7), 467-474; R. Moriera et al. Tetrahedron Lett. 1994, 35(38), 7107-7110; R. W. A. Luke, Tetrahedron Lett. 1996, 37(2), 263-266).

[0068] Alternatively compounds of Formula (III) can be synthesized according to the following scheme:



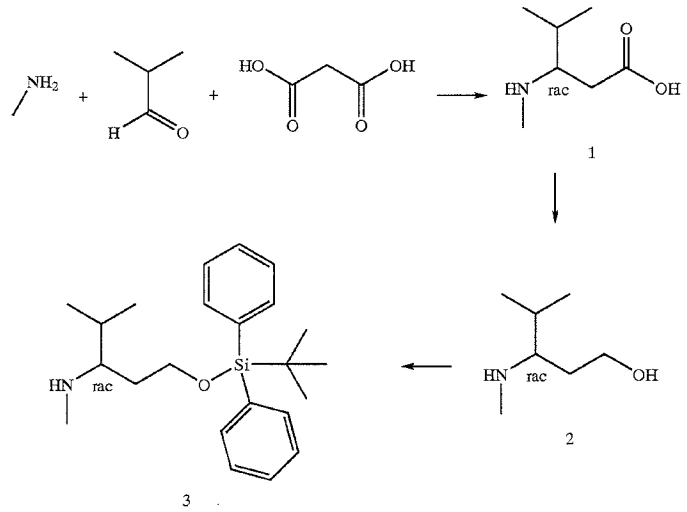
[0069] Building block (VI) of the following Formula:



[0070] can be steteoselectively synthesized using Evens reaction.

EXAMPLES

[0071]



Synthesis of N-methyl-β-R,S-valine (1)

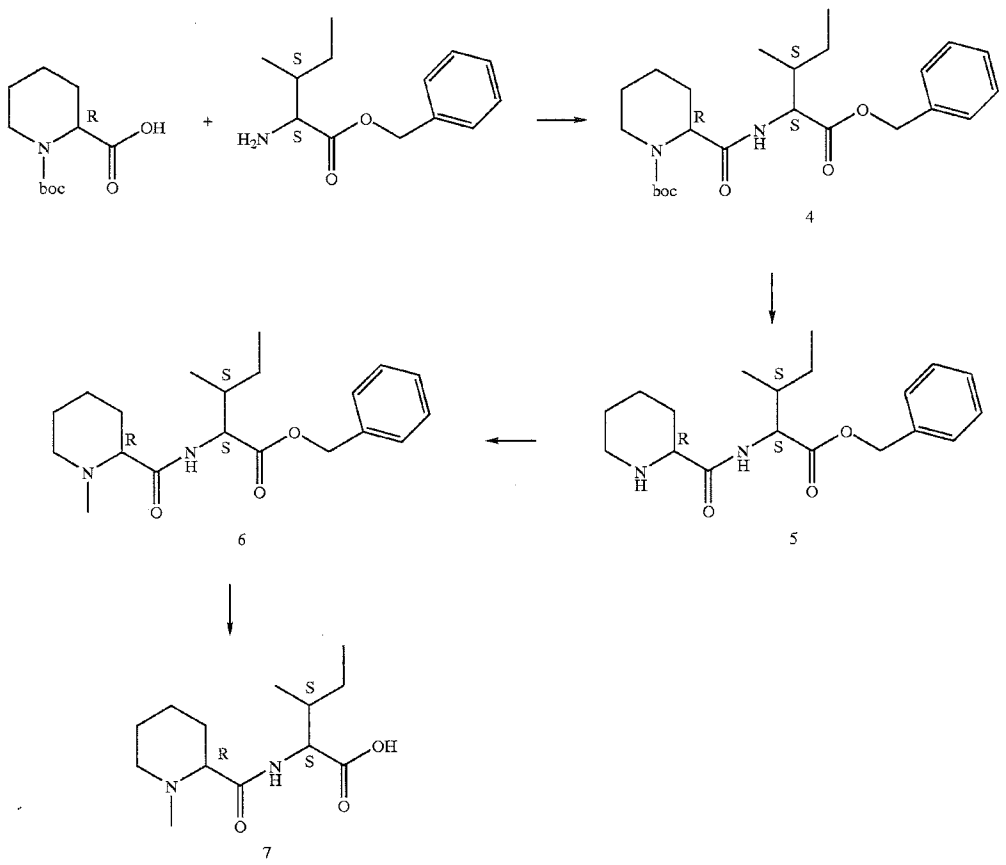
[0072] 58.8 ml (0.47 mol) of a 8M methylamine solution in ethanol are slowly dropped to a solution of 33.8 g isobutyric aldehyde (0.47 mol) in 200 ml ethanol while keeping the temperature in the flask below 5° C. Then 50 ml THF are added and the mixture is refluxed for 1 h. Then 48.91 g (0.47 mol) malonic acid is added in small portions and the mixture is refluxed for 5 h. After cooling to 25° C. the prcipitated is filtered off, washed with THF and dried under high vaccuum. Yield: 50.34 g N-methyl-□-R,S-valine. Mass spectroscopy: expected molecular mass 145.2; found: m/z $(M+H)^+$=146.1.

Synthese von N-Methyl-β-R,S-valinol (2)

[0073] 14.5 g (0.1 mol) N-methyl-β-R,S-valine in 135 ml dry THF are added slowly to 150 ml 1M lithiumaluminium hydrid in THF (0.15 mol) while keeping the temperature in the flask below 5° C. This mixture is refluxed for 4 h. Subseqently the mixture is stirred over night. The mixture is hydrolized with 4 ml 12% KOH and 4 ml water. The precipitate is filtered off and is extracted two times with 80 ml hot THF. The filtrates are combined and the solvent is removed under vaccuum. The resulting oil is destillled (bp.: 48° C./0.5 mbar). Yield: 8.28 g N-methyl-β-R,S-valinol. Mass spectroscopy: expected molecular mass 131.2; found: m/z $(M+H)^+$=132.2.

Synthesis of N-methyl-β-R,S-valinolyl-tert.-butyl-diphenyl-silylether (3)

[0074] 2 g N-Methyl-β-R,S-valinole (15.24 mmol) are solubilized in 20 ml dry dichlormethan together with 465.5 mg dimethylaminopyridin (3.81 mmol) and 2.66 ml triethylamine (19.05 mmol). To this solution 4.61 ml tert.-butyldiphenylsilylchloride (18 mmol) is added and the mixture is stirred over night. 20 ml Water and 20 ml dichlormethane are added. The water phase is extracted two times with dichlormethane and the combined organic phases are dried over sodium sulfat. The sodium sulfate is filtered of and the solvent is evaporated undeer vaccuum. The residual oil is purified using column chromatography (eluent: ethylacetat/ethanol=8:2). Yield: 3.94 g N-nethyl-β-R,S-valinolyl-tert-.butyldiphenylsilylether. Mass spectroscopy: expected molecular mass 369.6; found: m/z $(M+H)^+$=370.5.



Assembly of the dipeptide (R)-N-Boc-homoPro-(S, S)-Ile-OBzl (4)

[0075] 7 g 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (21.81 mmol) and 2.4 ml N-methylmorpholin (21.81 mmol) are added to a solution of 5 g (R)-N-Boc-homoprolin (21.81 mmol) in 40 ml dry DMF. After 10 minutes 7.21 g (S,S)-H-Ile-OBzl tosylat (18.32 mmol) and 2 ml N-methylmorpholin (18.32 mmol) are added. This mixture is stirred over night at 25° C. and then 40 ml ethylacetate are added. The organic layer is washed with saturated $NaHCO_3$. The aqueous layer is extracted two times with ethylacetate. The combined organic extracts are washed with saturated NaCl and dried over Na_2SO_4. The solvent is evaporated under vaccuum and the pure product appears. Yield 5.54 g (R)-N-Boc-homoPro-(S,S)-Ile-OBzl. Mass spectroscopy: expected molecular mass 432.6; found: m/z $(M+H)^+$=433.6

Boc-deprotection of (R)-N-Boc-homoPro-(S,S)-Ile-OBzl (5)

[0076] To a solution of (R)-N-Boc-HomoPro-(S,S)-Ile-OBzl in 60 ml dry THF is added 120 ml 4M HCl in dioxan while keeping the temperature in the flask below 5° C. After allowing the temperature to come to 20° C. the mixture is stirred for 5 h. The solvent is evaporated and can be used directly without further purification for the next step. Yield: 4.1 g (R)-H-homoPro-(S,S)-Ile-OBzl. Mass spectroscopy: expected molecular mass 332.5; found: m/z $(M+H)^+$=333.6.

Reductive amination of (R)-H-homoPro-(S,S)-Ile-OBzl (6)

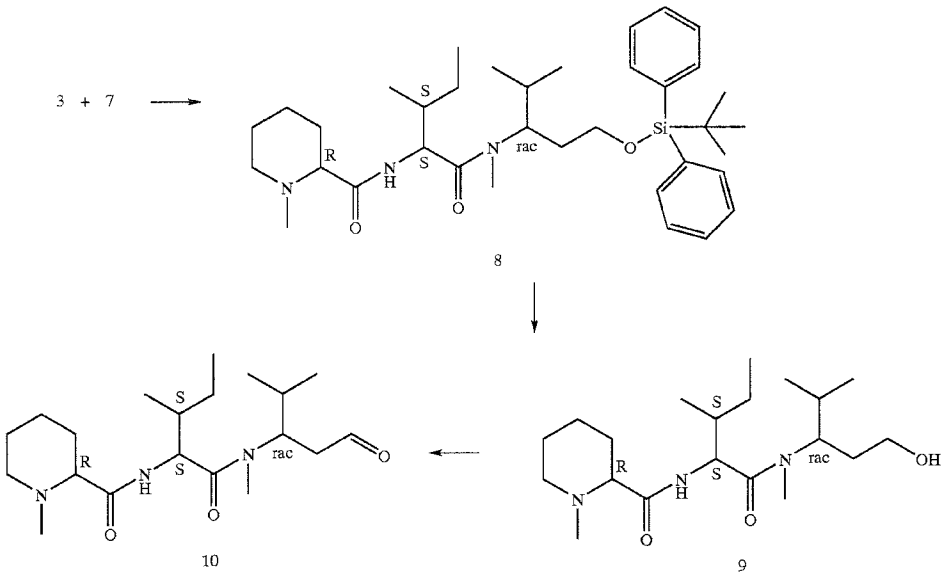
[0077] 10 ml of a 37% formaldehyde solution (123 mmol) is added to 4.1 g (R)-homoPro-(S,S)-Ile-OBzl (12.3 mmol) in 20 ml methanol. The pH is addjusted to 5-6 ewith acetic acid and 1.932 g sodium cyanoborhydride (30.75 mmol) is added in portions. The mixture is stirred for 16 h at 20° C. Subsequently the reaction is acidified with conc. HCl. The solvent is evaporated under vaccuum and water is added. The pH is addjusted to pH 12 with solide NaOH and the mixture is extracted three times with dichlormethan. The organic layer is dried with Na_2SO_4 and the solvent is evaporated. The resulting oil is evaporated by column chrommatography (eluent: ethylacetat:n-heptan=1:1). Yield: 3.9 g (R)-N-methyl-homoPro-(S,S)-Ile-OBzl. Mass spectroscopy: expected molecular mass 346.5; found: m/z $(M+H)^+$=347.4

Hydration of (R)-N-methyl-homoPro-(S,S)-Ile-OBzl (7)

[0078] To a solution of 3.9 g (R)-N-methyl-homoPro-(S, S)-Ile-OBzl (11.26 mmol) in 30 ml methanol, 1.2 g Pd (10%

C) are added. The flask is first flushed with N_2 and then 10 min with H_2. Two more h the suspension is stirred under a H2-ballone; then the catalyst is filtered through celite, and washed two times with methanol. The solvent is evaporated and the residual oil is lyophylized giving a white powder. Yield: 2.7 g (R)-N-methyl-homoPro-(S,S)-Ile-OH. Mass spectroscopy: expected molecular mass 256.4; found: m/z $(M+H)^+$=257.4

niumfluorid (1M in THF) (7.72 mmol) are added dropwise and the resulting mixture is stirred for 2 h at 20° C. Then 8 ml of water is added and the tetrahydrofuran is evaporated under vaccuum. The solution is neutralized and extracted five times with ethylacetat. The combined organic phases are extracted two times with saturated NaCl and dried over Na_2SO_4. The Na_2SO_4 is filtered off and the solvent is evaporated. The resulting product is pure enough for further



Coupling of (R)-N-methyl-homoPro-(S,S)-Ile-OH and N-methyl-β-R,S-valinolyl-tert.butyldiphenylsilylether (8)

[0079] To a solution of 3.522 g (R)-N-methyl-homoPro-(S,S)-Ile-OH (13.74 mmol) in 15 ml dry DMF, 2.104 g hydoxybenzotriazol (13.74 mmol) and 2.151 ml diisopropylcarbodiimide (13.74 mmol) are added. After 15 minutes stirring 4.232 g N-methyl-β-R,S-valinolyl-tert.butyldiphenylsilylether (11.45 mmol) is added and the mixture is stirred for 16 h at 20° C. The precipitated diisopropyl urea is filtered off and the solvent is evaporated under vaccuum. The residue is thoroughly stirred wit dichlomethane and the residual diisopropyl urea is filtered off. The dichlormethan solution is extracted with NaHCO3 and dryed subsequently with Na2SO4. After filtering off the Na2SO4 the solvent is evaporated under vaccuum. The residue is purified with preperative HPLC. (RP-C18, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 3.91 g. Mass spectroscopy: expected molecular mass 608.0; found: m/z $(M+H)^+$= 609.0.

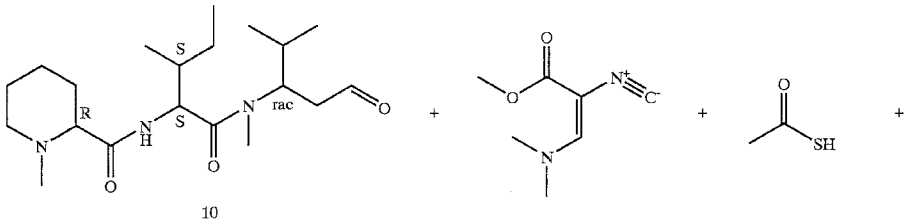
Deprotection of the tert.butyldiphenylsilyl protecting group of (8) (9)

[0080] 3.91 g Of compound 8 (6.43 mmol) are solubilized in 30 ml dry tetrahydrofuran and 2.223 ml tetrabutylammo-

transfromations. Mass spectroscopy: expected molecular mass 369.6; found: m/z $(M+H)^+$=370.5.

Swern-Oxidation of (9) (10)

[0081] A solution of 0.665 ml oxalylchloride (7.75 mmol) in 25 ml dry dichlormethan in a 250 ml flask is cooled to −70° C. under a N_2 atmosphere. Slowly 1.188 ml dimethylsulfoxide (16.73 mmol) in 5 ml dry dichlormethane is added in a way that the inner temperature is kept below −60° C. and the resulting mixture is stirred for 30 minutes at −70° C. Then a solution (6 ml) of (9) (6.43 mmol) in dichlormethane is added in a way that the inner temperature is kept below −60° C. After stirring for further 30 minutes 4.459 ml triethylamin (32.17 mmol) are added at −70° C. Once the flask reached 20° C., 15 ml water are added and further 10 minutes are stirred. The aqueous phase is extracted two times with dichlormethan. The combined orgaic phases are dryed over Na_2SO_4, the Na_2SO_4 is filtered off and the solvent is evaporated. The resulting product is pure enough to be used in the next step. Mass spectroscopy: expected molecular mass 367.6; found: m/z $(M+H)^+$=368.5.



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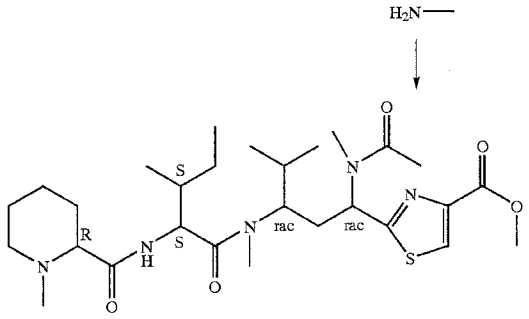
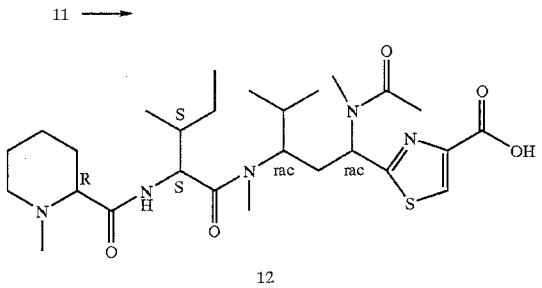


11

Thiazolsynthesis (11)

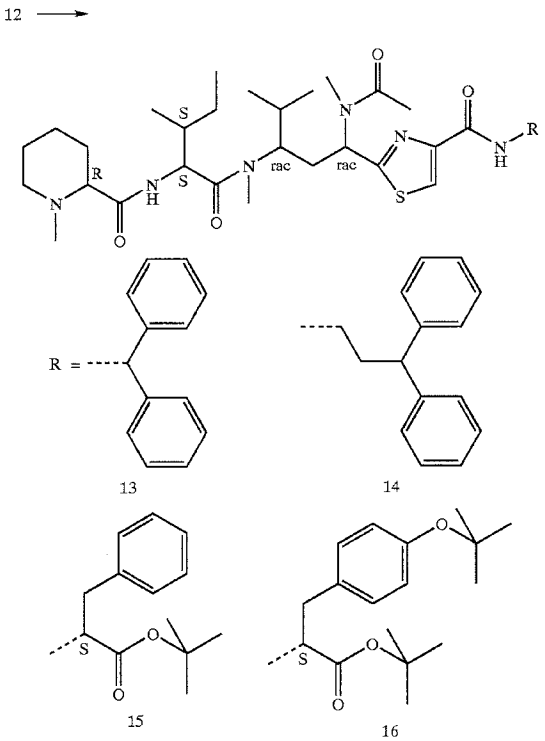
[**0082**] 0.695 ml Methylamin solution (33% in ethanol) (7.72 mmol) are added to (10) in 20 ml dry methanol and stirred for 1 h at 20° C. 991.3 mg 3-Dimethylamino-2-isocyano-acrylacidmethylester (6.43 mmol) and 0.457 ml thioacetic acid (6.43 mmol) are added and stirred for 16 h at 20° C. The slvent is evaporate under vaccuum and the residue is purified by preperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 1.294 g. Mass spectroscopy: expected molecular mass 565.8; found: m/z $(M+H)^+$=566.7



12

Saponification (11) (12)

[**0083**] To a solution of 1.294 g (11) (2.29 mmol) in 20 ml THF 220 mg LiOH (9.16 mmol) in 20 ml wate rare added and stired for 16 h at 20° C. This mixture is neutralized with 2N HCl. The solvent is evaporated under reduced pressure and the residue is purified with preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 1.14 g. Mass spectroscopy: expected molecular mass 551.8; found: m/z $(M+H)^+$=552.7



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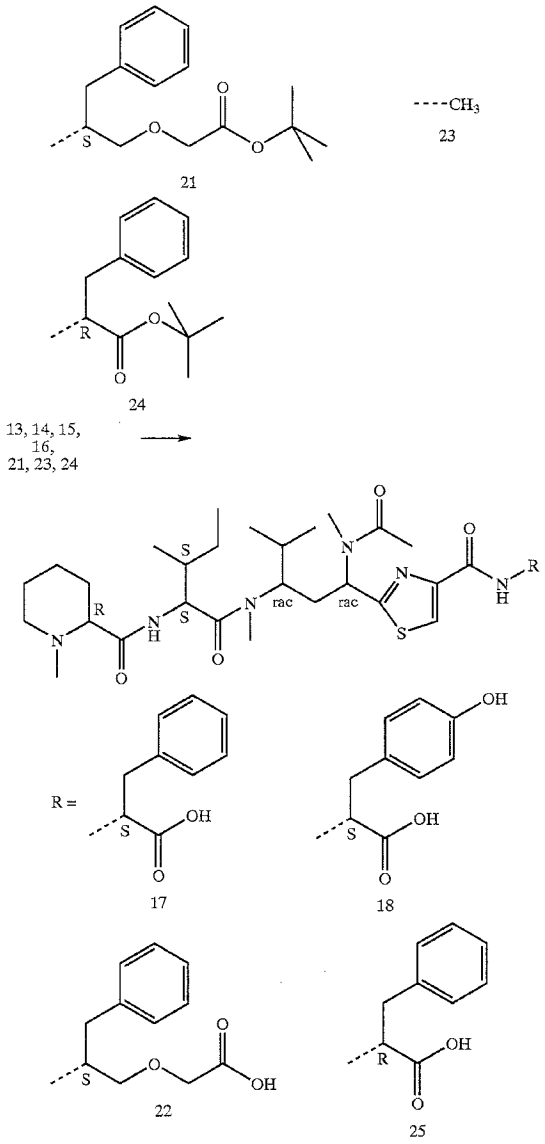


Coupling of (12) and □-aminodiphenylmethane (13)

[0084] To a solution of 49.5 mg (12) (0.09 mmol) in 3 ml dry DMF 18.6 mg 6-chlorhydroxybenzotriazole (0.11 mmol) and 0.014 ml diisopropylcarbodiimide (0.11 mmol) are added. This mixture is stirred for 15 minutes at 20° C. and 0.062 ml α-aminodiphenylmethan (0.36 mmol) are added. This mixture is stirred over night at 20° C., then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 35 mg. Mass spectroscopy: expected molecular mass 717.0; found: m/z $(M+H)^+$= 718.1

Coupling of (12) and 3,3-diphenylpropylamine (14)

[0085] To a solution of 49.5 mg (12) (0.09 mmol) in 3 ml dry DMF 18.6 mg 6-chlorhydroxybenzotriazole (0.11 mmol) and 0.014 ml diisopropylcarbodiimide (0.11 mmol) are added. This mixture is stirred for 15 minutes at 20° C. and 76 mg 3,3-diphenylpropylamin (0.36 mmol) are added. This mixture is stirred over night at 20° C., then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 35 mg. Mass spectroscopy: expected molecular mass 745.0; found: m/z $(M+H)^+$=746.1.

Coupling of (12) and S-phenylalanine tert.butylester (15)

[0086] To a solution of 49.5 mg (12) (0.09 mmol) in 3 ml dry DMF 18.6 mg 6-chlorhydroxybenzotriazole (0.11 mmol) and 0.014 ml diisopropylcarbodiimide (0.11 mmol) are added. This mixture is stirred for 15 minutes at 20° C. and 24.3 mg S-phenylalanine tert.butylester (0.11 mmol) are added. This mixture is stirred over night at 20° C., then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 35 mg. Mass spectroscopy: expected molecular mass 755.0; found: m/z $(M+H)^+$= 756.2.

Coupling of (12) and S-tyrosin-O-tert.-butylether-tert.-butylester (16)

[0087] To a solution of 49.5 mg (12) (0.09 mmol) in 3 ml dry DMF 18.6 mg 6-chlorhydroxybenzotriazole (0.11 mmol) and 0.014 ml diisopropylcarbodiimide (0.11 mmol) are added. This mixture is stirred for 15 minutes at 20° C. and 32.3 mg S-tyrosin-O-tert.-butylether-tert.-butylester (0.11 mmol) are added. This mixture is stirred over night at 20° C., then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 35 mg. Mass spectroscopy: expected molecular mass 827.1; found: m/z $(M+H)^+$=828.0.

Deprotection of (15) (17)

[0088] To a solution of 26 mg (15) (0.034 mmol) 2 ml dry dichlormethan 2 ml trifluoracetic acid are added. The mixture is stirred for 1 h and the solvent is evaporated under the addition of n-heptan. The product is pure. Yield: 20 mg. Mass spectroscopy: expected molecular mass 698.9; found: m/z $(M+H)^+$=699.5.

Deprotection of (16) (17)

[0089] To a solution of 26 mg (16) (0.034 mmol) 2 ml dry dichlormethan 2 ml trifluor acetic acid are added. The mixture is stirred for 1 h and the solvent is evaporated under the addition of n-heptan. The product is pure. Yield: 18 mg. Mass spectroscopy: expected molecular mass 714.9; found: m/z (M+H)+715.5.

Coupling of benzyloxycarbonyl-S-phenylalaninol and bromo aceticacid-tert.-butyl-ester (19)

[0090] To a solution of 1.141 g benzyloxycarbonyl-S-phenylalaninol (4 mmol) in 20 ml dry THF 160 mg sodi-

umhydrid dispersion (60% in mineral oel) are added. After end of H_2 evolution 1.182 ml bromo acetic acid tert.-butylester (8 mmol) are added and the mixture is stirred for 48 h at 20° C. The solvent is evaporated under reduced pressure and the product is purified with preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetci acid). Yield: 805 mg. Mass spectroscopy: expected molecular mass 399.5; found: m/z $(M+H)^+$=400.3

Cbz-deprotection of (19) (20)

[0091] To a solution of 805 mg (19) (2.02 mmol) in 15 ml methanol, 800 mg Pd (10% C) are added. The flask is first flushed with N_2 and then stirred 16 h under H2 atmosphere (2 H2 ballons). The catalyst is filtered through celite and washe several times with methanol. The solvent is evaporated. Yield: 482 mg. Mass spectroscopy: expected molecular mass 265.4; found: m/z $(M+H)^+$=266.3.

Coupling of (12) and (20) (21)

[0092] To a solution of 49.5 mg (12) (0.09 mmol) in 3 ml dry DMF 16.8 mg hydroxybenzotriazol hydrate (0.11 mmol) and 0.014 ml diisopropylcarbodiimid (0.11 mmol) are added. After stirring for 15 minutes at 20° C. 29.2 mg (20) (0.11 mmol) are added. After stirring over night at 20° C. the solution is filtered and the residue is pruified by HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 22 mg. Mass spectroscopy: expected molecular mass 799.1; found: m/z $(M+H)^+$= 800.2.

Deprotection of (21) (22)

[0093] To a solution of 22 mg (21) (0.028 mmol) in 2 ml dry dichlormethan 2 ml trifluoracetic acid are added. This mixture is stirred for 1 h at 20° C. and the solvent is evaporated upon addition of n-heptan. The product is pure. Yield: 16 mg. Mass spectroscopy: expected molecular mass 757.0; found: m/z $(M+H)^+$=758.2.

Coupling of (12) and methylamin (23)

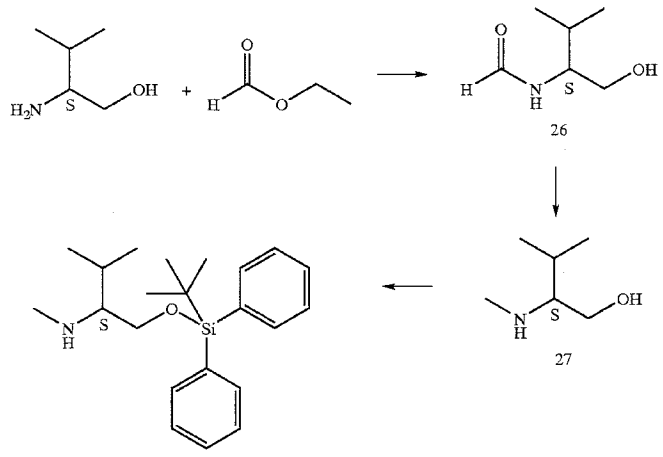
[0094] To a solution of 49.5 mg (12) (0.09 mmol) in 3 ml dry DMF 18.6 mg 6-chlorhydroxybenzotriazole (0.11 mmol) and 0.014 ml diisopropylcarbodiimide (0.11 mmol) are added. This mixture is stirred for 15 minutes at 20° C. and 0.22 ml methylamin solution (2M in THF) (0.44 mmol) are added. This mixture is stirred over night at 20° C., then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 35 mg. Mass spectroscopy: expected molecular mass 564.8; found: m/z $(M+H)^+$= 565.7.

Coupling of (12) and R-Phenylalanintert.butylester (24)

[0095] To a solution of 49.5 mg (12) (0.09 mmol) in 3 ml dry DMF 18.6 mg 6-chlorhydroxybenzotriazole (0.11 mmol) and 0.014 ml diisopropylcarbodiimide (0.11 mmol) are added. This mixture is stirred for 15 minutes at 20° C. and 24.3 mg R-phenylalanine tert.butylester (0.11 mmol) are added. This mixture is stirred over night at 20° C., then the solution is filtered and the solvent is evaporated under vaccum. The residue is purified by perperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 35 mg. Mass spectroscopy: expected molecular mass 755.0; found: m/z $(M+H)^+$= 756.2.

Deprotection of (24) (25)

[0096] To a solution of 23 mg (24) (0.03 mmol) in 2 ml dry dichlormethan 2 ml trifluoracetic acid are added. The mixture is stirred for 1 h and the solvent is evaporated under the addition of n-heptan. The product is pure. Yield: 18 mg. Mass spectroscopy: expected molecular mass 698.9; found: m/z $(M+H)^+$=699.5.



Synthesis of N-formyl-S-valinol (26)

[0097] 10 g S-Valinol (97 mmol) are dissolved in 50 ml ethylformiat and refluxed for 1 h. The solvvent is evaporated and the rsidue is destilled under vacuum (bp.: 153° C./0.5 mbar). Yield: 8.4 g. Mass spectroscopy: expected molecular mass 131.2; found: m/z $(M+H)^+$=132.3

Synthesis of N-methyl-S-valinol (27)

[0098] To a solution of 5.7 g lithiumaluminiumhydrid (150 mmol) in 200 ml dry THF, 8.4 g N-formyl-S-valinol (64 mmol) dissolved in 40 ml dry THF are added slowly and stirred for 16 h at 20° C. In several portions 30 g sodium sulfat decahydrat and 18 ml water are added and furthermore stirred for 3 h at 20° C. The solids are filtered off and the solvent is evapuorated under vaccuum. The residual material is purified by destillation (bp.: 93° C./54 mbar). Yield: 3.7 g. Mass spectroscopy: expected molecular mass 1.17.2 found: m/z $(M+H)^+$=118.1.

Synthesis of N-methyl-S-valinolyl-tert.butyldiphenylether (28)

[0099] To a solution of 1.64 g N-methyl-S-valinol (14 mmol) in 10 ml dry dichlormethane 427 mg dimethylaminopyridine (3.5 mmol) and 2.44 ml triethylamin (17.5 mmol) are added. Then 4.3 ml tert.butyldiphenylsilyl chloride are added and 16 h stirred at 20° C. Then 10 ml water and THF are added and the phases are seperated. The aqueous phase id extracted two times with dichlormethane. The combined organic phases are dried ove Na_2SO_4, subsequently the solvent is evaporated. The residue is purified by column chromatography (eluent: ethylacetat/ethanol= 8:2). Yield: 3.16 g. Mass spectroscopy: expected molecular mass 355.6 found: m/z (M+H)+366.6

Coupling of (R)-N-methyl-homoPro-(S,S)-Ile-OH and N-methyl-S-valinolyl-tert.butyldiphenylsilylether (29)

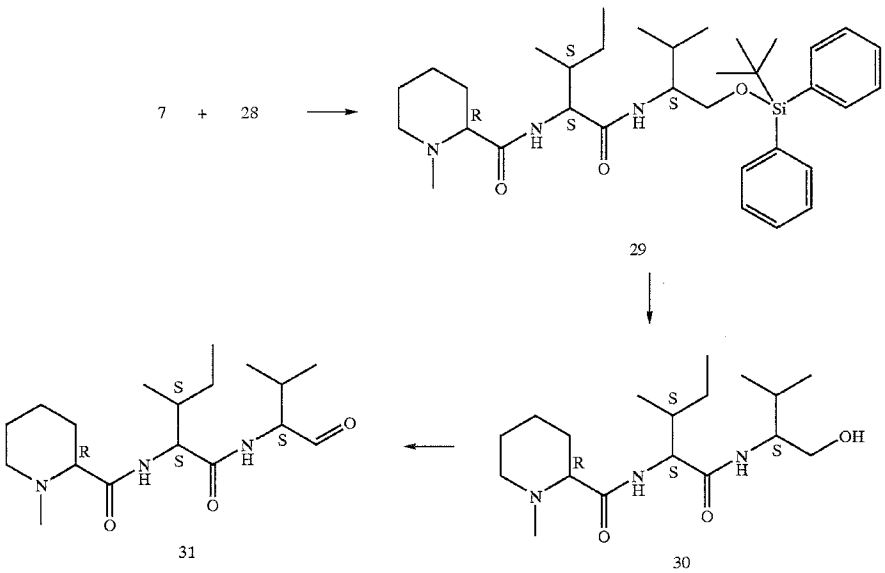
[0100] To a solution of 1.54 g (R)-N-methyl-homoPro-(S,S)-Ile-OH (6 mmol) in 10 ml dry DMF, 1.02 g 6-chlorohydroxybenzotriazol (6 mmol) and 0.939 ml diisopropylcarbodiimid (6 mmol) are added. The mixture is stirred for 15 minutes and 2.56 g N-ethyl-S-valinolyl-tert.butyldiphenylether (7.2 mmol) are added and stirred for 16 h at 20° C. Then the solvent is evaporated ubder vacuum and the residue is purified by preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 1.06 g. Mass spectroscopy: expected molecular mass 593.9 found: m/z $(M+H)^+$=594.8.

Cleavage of the tert.-butyldiphenylsilyl protecting group of (29) (30)

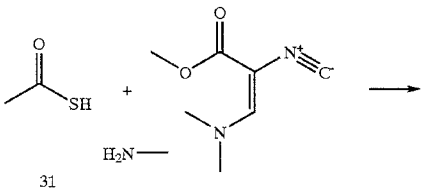
[0101] To a solution of 1.06 g (29) (1.79 mmol) in 10 ml dry THF a solution of 2.15 ml tetrabutylammoniumfluorid (1M Lösung in THF) (2.15 mmol) is added. The mixture is stirred for 16 h at 20° C. and then hydrolysed upon addition of 3 ml water. The organic solvent is evaporated and the aqueous phase is extracted five times with ethylacetat. The combined organic phases are washed with staturated NaCl and dried over Na_2SO_4. After filtration of Na_2SO_4 the solvent is evaporated. Yield: 1.05 g (some residual silyl is remaining). Mass spectroscopy: expected molecular mass 355.5 found: m/z $(M+H)^+$=356.5.

Swern-Oxidation of (30) (31)

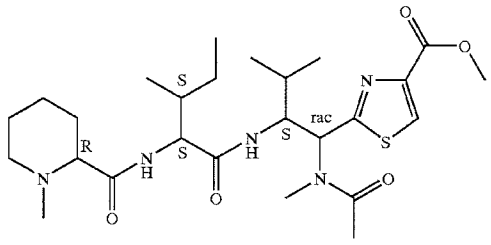
[0102] 0.316 ml Oxalylchlorid (1.98 mmol,) are solubilized in 3 ml dry dichlormethan in a 100 ml flask under N_2 atmosphere and cooled to −70° C. To this solution 0.305 ml



dimethylsulfoxid (4.29 mmol) in 0.6 ml dichlormethan are added slowly (evolution of gas, keep the temperatur below −60° C.) and stirring continues ofr 30 minutes. A solution of 587 mg (30) (1.65 mmol) in 2 ml dichlormethan is added while keeping the temperature below −60° C. and stirring for 30 minutes. Then 1.146 ml triethylamin (8.25 mmol) is added. The mixture is allowed to come to 20° C. and then 10 ml water are added and the mixture is stirred for another 10 minutes. The aqueous phase is extracted two times with dichlormethan. The combined organic layers are dried with Na_2SO_4. After filtering off the Na_2SO_4 the solvent is evaporated. Yield: 636 mg. Mass spectroscopy: expected molecular mass 353.5 found: m/z $(M+H)^+$=354.5.



31

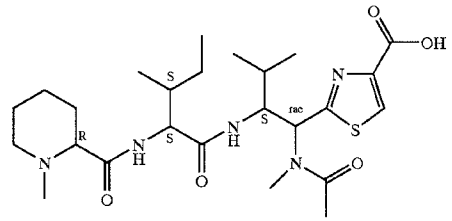


32

Thiazolsynthesis (32)

[**0103**] Mg (31) (1.15 mmol) and 0.173 ml methylamin (33% in ethanol) (1.38 mmol) in 3 ml dry methanol are stirred for 1 h at 20° C. 185 mg 3-Dimethylamino-2-isocyano-acrylciacidmethylester (1.2 mmol) and 0.086 ml thioacetic acid (1.2 mmol) are added and stirred for 16 h at 20° C. The solvent is evaporated and the residue is purified with preperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 150 mg. Mass spectroscopy: expected molecular mass 551.8; found: m/z $(M+H)^+$=552.7.

32 ⟶

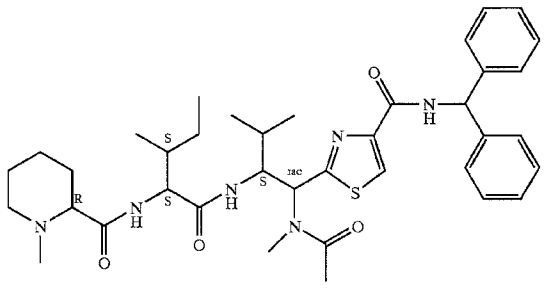


33

Saponification of (32) (33)

[**0104**] To a solution of 61 g (32) (0.11 mmol) in 2 ml THF, 10.6 mg LiOH (0.44 mmol) in 2 ml water is added and stirred for 16 h at 20° C. The mixture is neutralized with 2N HCl. The solvent is evaporated and the residue is purified with preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 50 mg. Mass spectroscopy: expected molecular mass 537.7; found: m/z $(M+H)^+$=538.7.

33 ⟶



34

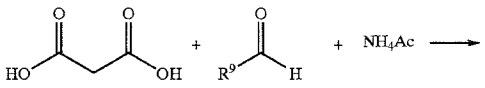
Coupling of (33) and α-aminodiphenylmethane (34)

[**0105**] To a solution of 49.5 mg (33) (0.093 mmol) in 3 ml dry DMF, 14.2 mg hydroxybenzotriazol (0.093 mmol) and 0.012 ml diisopropylcarbodiimid (0.093 mmol) are added and stirred for 15 minutes at 20° C. 0.064 ml α-aminodiphenylmethan (0.372 mmol) is added and is stirred over night. The mixture is filtered and evaporated and the residue is purified by preparative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid). Yield: 30 mg. Mass spectroscopy: expected molecular mass 703.0; found: m/z $(M+H)^+$=704.1.

General Procedure for the Synthesis of Thiazoles

[**0106**] 1 Mmol of the carbonyl compound (IX) is solubilized in 3 ml dry THF gelost under N_2 atmosphere and 1 mmol borontrifluorid etherat are added. After 10 min 1 mmol of isocyanide (VIII) and 1 mmol of thioacarboxylic acid (VII) are added and stirred for 72 h. Water is added and optinally filtered through celite. The solvent is evaporated under vacuum. The residue is solubilized in ethylacetate. The organic phase is washed two times with water. After drying the organic phase over Na_2SO_4 the slvent is evaporated. The residue is purified by preperative HPLC (reversed phase-C18-phase, eluent methanol+0.5% acetic acid/water+0.5% acetic acid).

[**0107**] Compounds of Formula (IX) can be synthesized for example by a α-aminoalkylation of isobutyric aldehyd, ammoniumacetat or a primary amine or amine hydrochlorid and malonic acid:



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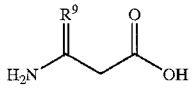
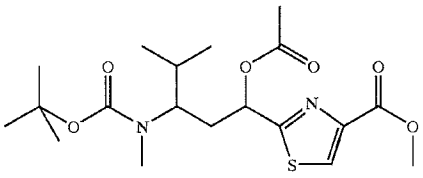


[0108] The resulting β-amino acid can be subsequently N-alkylated (e.g. by reductive amination) and protected (e.g. t-butyloxycarbonyl, Boc). Then the carboxylic acid group is transformed to the aldehyde (e.g. by reduction to the alkohol by $LiAlH_4$ and subsequent Swern oxidation to the aldehyde; see for example R. C. Larock, Comprehensive Organic Transformations, VCH Publishers, New York, 1989). Alternatively the β-aminoacid can be synthesized by a Arndt-Eistert procedure starting from valine.

Example 35

[0109]

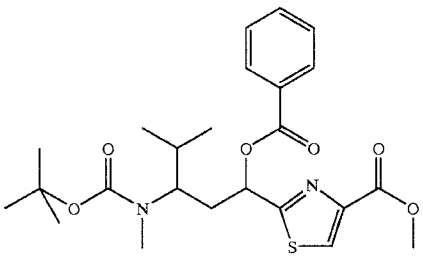


[0110] $C_{19}H_{30}N_2O_6S$ (414.5248)

[0111] MS (ESI): 415 [M+H]

Example 36

[0112]

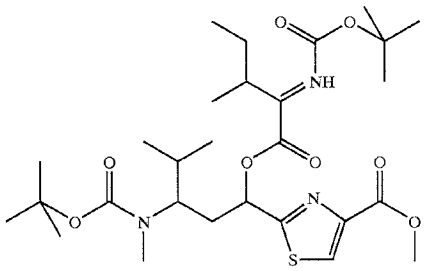


[0113] $C_{24}H_{32}N_2O_6S$ (476.5964)

[0114] MS (ESI): 477 [M+H]

Example 37

[0115]

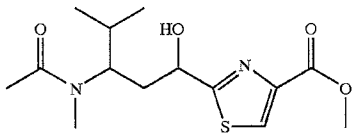


[0116] $C_{28}H_{47}N_3O_8S$ (58537661)

[0117] MS (ESI): 586 [M+H]

Example 38

[0118] A compound from example 35 (0.1 mmol) is stirred in 2 ml dichlormethan (DCM) and 0.1 ml trifluoracetic acid (TFA) for 1 h at 20° C. The liquides DCM/TFA are evaporated and the residue is purified by HPLC.

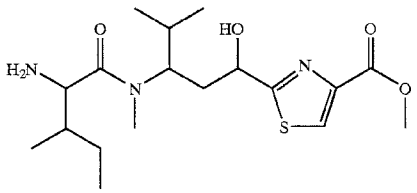


[0119] $C_{14}H_{22}N_2O_4S$ (314.4064)

[0120] MS (ESI): 315 [M+H]

Example 39

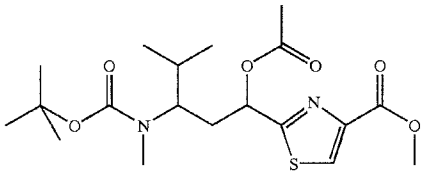
[0121] The compound from example 37 (0.1 mmol) is dissolved in 2 ml Ddchlormethan (DCM) and 0.1 ml trifluoracetic acid (TFA) is added and stirred for 1 h at 20° C. The liquides DCM/TFA are evaporated and the residue is purified by HPLC.



[0122] $C_{18}H_{31}N_3O_4S$ (385.5295)

[0123] MS (ESI): 386 [M+H]

[0124] Bispiel 40:

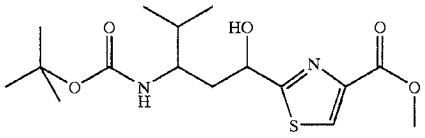


[0125] $C_{18}H_{28}N_2O_6S$ (400.4977)

[0126] MS (ESI): 401 [M+H]

Eample 41

[0127] 1 mmol of the compound from example 40 in 1 ml methanol is stirred with 1 ml 4 M ammonia solution in methanol for 2 h at 20° C. Tsolvent is evaporated under vacuum.



[0128] $C_{16}H_{26}N_2O_5S$ (358.4600) MS (ESI): 381 [M+Na]

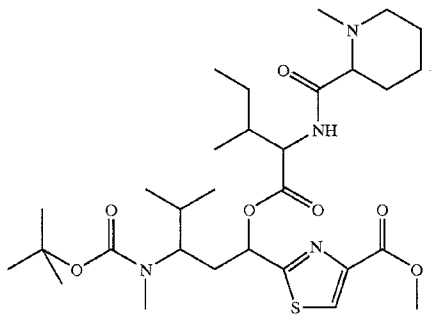
Example 42 and 43

Ester Coupling of Hydroxythiazols (Example 41) and Dipeptide (7) and Subsequent Transacylation

[0129] To 2 Mmol (512 mg) 3-methyl-2-[(1-methyl-piperidin-2-carbonyl)-amino]-pentanoic acid (7) in 5 ml dry dichlormethan is added 2 mmol (252 mg) N,N'-diisopropylcarbodiimide (DIC) in 2.5 ml DCM and 0,2 mmol (24 mg) DMAP in 2.5 ml DCM under N_2 atmosphere at 0° C. The mixture is stirred 5 minutes at 0° C. 1 mmol (372 mg) 2-[3-(tert.-butoxycarbonyl-methylamino)-1-hydroxy-4-methyl-pentyl]-thiazole-4-carboxylic acid methylester (example 41) is dissolved in 5 ml DCM and slowly added via syringe. The mixture is stirred 4 h at 20° C. The mixture concentrated in vacuum and the precipitated urea is filtered off. To the filtrate is added 1 ml of trifluoracetic acid and 1 h stirred at 20° C. and the solvents are evaporated under vacuum. The residue is dissolved in 1 ml dry dichlormethan and 1 ml triethylamin is added and 1 h stirred at 20° C. The solvent is evaporated under vacuum. The rearranged coupling product is purified by HPLC.

2-(3-(tert.-butoxycarbonyl-methyl-amino)-4-methyl-1-{3-methyl-2-[(1-methyl-piperidin-2-carbonyl)-amino]-pentanoyloxy}-pentyl)-thiazol-4-carboxylic acid methylester (42)

[0130]

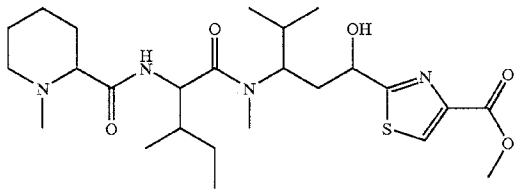


[0131] $C_{30}H_{50}N_4O_7S$ (610,82)

[0132] MS (ESI): 611 [M+H]; 633 [M+Na]

2-[1-Hydroxy-4-methyl-3-(methyl-{3-methyl-2-[(1-methyl-piperidin-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl]-thiazol-4-carboxylic acid methyl-ester (43)

[0133]



[0134] $C_{25}H_{42}N_4O_5S$ (510,70)

[0135] MS (ESI): 511 [M+H]; 533 [M+Na]

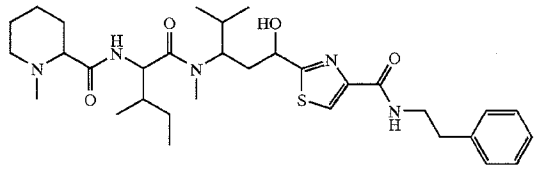
Example 44 and 45

Reaction of (43) and Phenylethylamine and Subsequent Acetylation

[0136] 0.14 Mmol (72 mg) 2-[1-hydroxy-4-methyl-3-(methyl-{3-methyl-2-[(1-methylpiperidine-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl]-thiazol-4-carboxylic acid methylester (43) are stirred with 100 µl phenylethylamine for 12 h at 20° C. The reaction mixture is filtered thhrough a plug of silica gel and washed with ethylacetate. The mixture is evaporated to dryness and 40 µl acetic acid anhydride and 10 µl pyridine are added. The mixture is stirred for 2 h at 20° C. A third of the reaction mixture is purified with a analytical HPLC.

1-methyl-piperidin-2-carbonsaure-[1-({1-[2-hydroxy-2-(4-phenethylcarbamoyl-thiazol-2-yl)-ethyl]-2-methyl-propyl}-methylcarbamoyl)-2-methyl-butyl]-amide (44)

[0137]

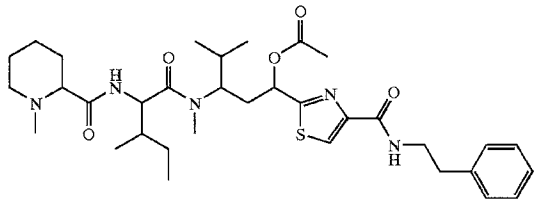


[0138] $C_{32}H_{549}N_5O_4S$ (599,84)

[0139] MS (ESI): 600 [M+H]; 622 [M+Na]

Acetic acid 4-methyl-3-(methyl-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-1- (4-phenethylcarbamoyl-thiazol-2-yl)-pentylester (45)

[0140]

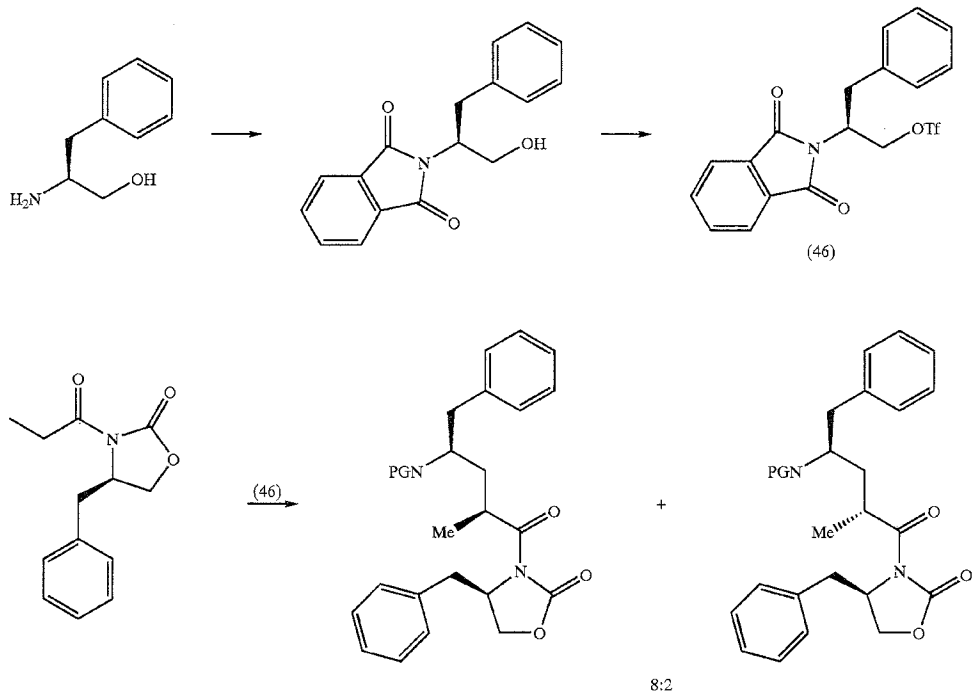


[0141] $C_{34}H_{51}N_5O_5$ S (641,88)

[0142] MS (ESI): 642 [M+H]; 664 [M+Na]

Synthesis of Building Block (VI) According to Evans-Procedure

[0143]



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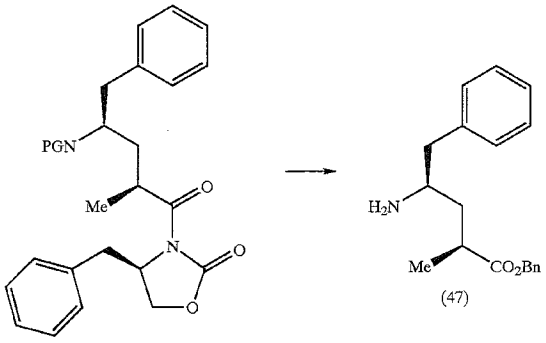


(47)

(2S)-2-Phthalimido-3-phenyl-propanol

[0144] To L-phenylalaninol (1.0 g, 6.61 mmol) and Na_2CO_3 (1.05 g, 9.92 mmol) in a 1:1 mixture of THF (10 mL) and H_2O (10 mL) N-carbethoxyphthalimide (1.74 g, 7.94 mmol) is added and stirred 4 h at 20° C. To this reaction mixture ethylacetate (20 mL) is added. The aqueous phase is extracted two times with 15 mL ethylacetate and the combined organic phases are washed with saturated NaCl, dried with Na_2SO_4 and the solvent is evaporated under vacuum. The product is purified with column chromatography using 2% MeOH in CH_2Cl_2. Yield: 1.41 g (76%); MS (ESI) 282 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82-7.76 (m, 2H), 7.73-7.66 (m, 2H), 7.24-7.12 (m, 5H), 4.70-4.58 (m, 1H), 4.12-4.02 (m, 1H), 3.98-3.88 (m, 1H), 3.20 (d, J=12.5 Hz, 2H), 2.80-2.72 (m, 1H).

S)-1-Trifluoromethanesulfonyl-2-phthalimido-3-phenyl propanoat

[0145] To a solution of (2S)-2-phthalimido-3-phenylpropanole (0.42 g, 1.49 mmol) in dry CH_2Cl_2 (5 mL), pyridin (146 μL, 1.79 mmol) is added at −78° C. and stirred for 20 minutes. To this mixture 3 min trifluoromethansulfonic acid anhydrid (264 μL, 1.57 mmol) is added in between 3 minutes and stirred for 1 h at −78° C. The reaction mixture is quentched with 3 ml saturated NaCl. The aqueous phase is extracted with 5 mL of CH_2Cl_2, the combined organic phases are washed with 5 ml saturated NaCl gewaschen, dried with Na_2SO_4 and the solvent is evaporated. The product is purified with column chromatography using 20% ethylacetat in hexen. Yield: 0.41 g (66%). MS (ESI) 414 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84-7.77 (m, 2H), 7.75-7.68 (m, 2H), 7.28-7.14 (m, 5H), 5.18 (t, J=13.0 Hz, 1H), 5.00-4.85 (m, 1H), 4.55-4.30 (m, 1H), 3.40-3.25 (m, 2H).

[0146] Evans Alkylation:

[0147] (4R)-3-propanoyl-4-benzyl-2-oxazolidinone (0.100 g, 0.43 mmol) is dissolved in 2 ml dry THF in an argon atmosphere and subsequently cooled to −40° C. LiHMDS (1M/THF) (0.47 mL, 0.47 mmol) is added and stirred for 45 minutes. (2S)-1-Trifluoromethansulfonyl-2-phthalimido-3-phenylpropanoate (0.266 g, 0.64 mmol) in dry THF (2 mL) is added. The mixture is stirred for 4 h at −40° C. and subsequently quentched with 3 ml saturated NaCl. The aqueous phase is extracted 2 times with 5 ml ethylacetate. The combined organic phases are washed with 3 ml saturated NaCl, dried with Na_2SO_4 and the solvent is evaporated under vacuum. The product is purified with column chromatograpgy using 25% ethylacetate in hexen. Yield: 0.149 g (70%). The diastereomers can be separated using preperative TLC. The wanted diastereomer is formed in excess: 8:2.

(2'S,4'R,4R,)-3-(2'Methyl-4'phthalimido-5'phenyl pentanoyl)-4-benzyl-1,3-oxazolidin-2-one (major product)

[0148] MS (ESI): 497 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (t, J=8.5 Hz, 2H), 7.63 (t, J=8.4 Hz, 1H), 7.55 (t, J=8.4 Hz, 1H), 7.42 (d, J=8.5 Hz, 2H), 7.37-7.22 (m, 6H), 7.10 (d, J=8.6 Hz, 2H), 5.08 (q, J=9.6 and 16.1 Hz, 1H), 4.56-4.42 (m, 2H), 4.20-4.00 (m, 4H), 3.45 (dd, J=10.7 and 16.1 Hz, 1H), 3.12-2.98 (m, 2H), 2.34 (dd, J=12.8 and 13.9 Hz, 1H), 1.62 (d, J=8.6 Hz, 3H).

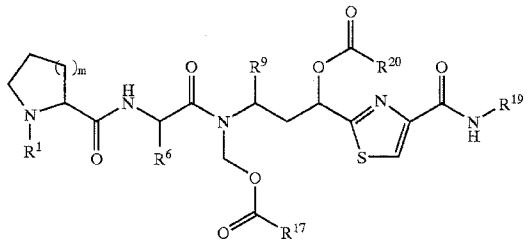
(2'R,4'R,4R,)-3-(2'Methyl-4'phthalimido-5'phenyl pentanoyl)-4-benzyl-1,3-oxazolidin-2-one (minor product)

[0149] MS (ESI): 497 [M+H]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (d, J=8.6 Hz, 1H), 7.76 (d, J=8.5 Hz, 1H), 7.63 (t, J=8.6 Hz, 1H), 7.53 (t, J=8.5 Hz, 1H), 7.40-7.20 (m, 10H), 5.10 (q, J=7.5 and 15.0 Hz, 1H), 4.94-4.84 (m, 1H), 4.54-4.42 (m, 1H), 4.36-4.08 (m, 4H), 3.46-3.30 (m, 2H), 3.12 (dd, J=9.6 and 11.8 Hz, 1H), 2.88 (dd, J=9.5 and 12.8 Hz, 1H), 1.00 (d, J=9.6 Hz, 3H).

[0150] Cleavage of the oxazolidinons: Evans et. al., J. Am. Chem. Soc. 1982, 104, 1737-1739.

[0151] Deprotection of the phthalimids: using hydrazine/EtOH at 20° C.: Sasaki, T. et. al., J. Org. Chem. 1978, 43, 2320; Khan, M. N. et. al., J. Org. Chem. 1995, 60, 4536.

[0152] According to the herein disclosed synthetic procedures also the following tubulysin derivatives where synthesized:



[0153] The following residues where used:

[0154] m=0, 1, 2, 3;

[0155] R^1 methyl, ethyl;

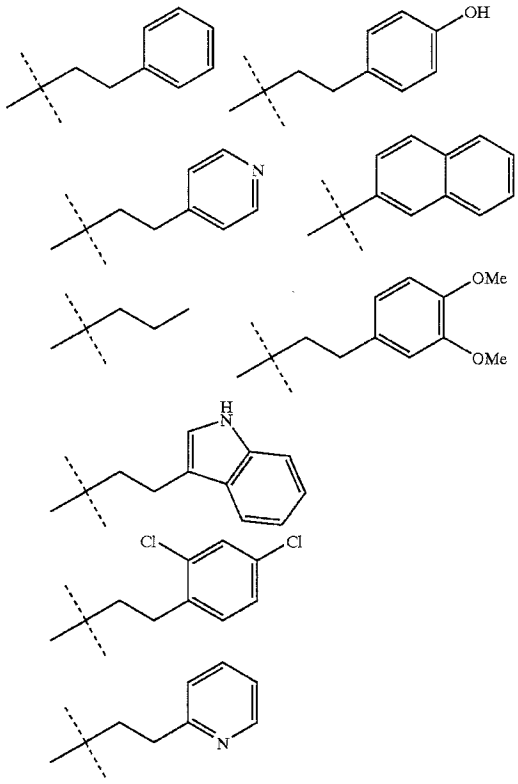
[0156] R^6=isopropyl, isobutyl, ethyl, cyclopropyl, CH_2-cyclopropyl, $CH(CH_3)CH_2CH_3$;

[0157] R^9=isopropyl, trifluormethyl, chlormethyl, isobutyl, ethyl, cyclopropyl, CH_2-cyclopropyl, $CH(CH_3)CH_2CH_3$, cyclopentyl, cyclohexyl;

[0158] R^{17}=methyl, ethyl, propyl, isopropyl, butyl, isobutyl, $CH{=}C(CH_3)$, cyclopropyl, cyclobutyl, cyclohexyl;

[0159] R^{20}=methyl, ethyl, propyl, isopropyl, phenyl;

[0160] R^{19}=



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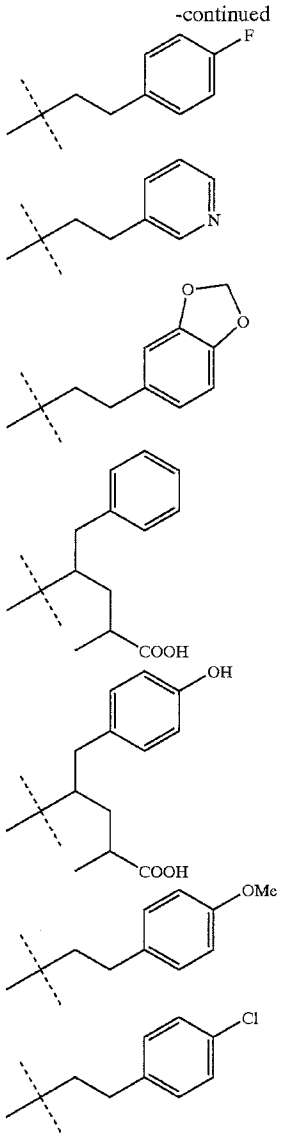
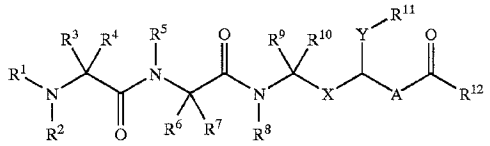


1-18. (canceled)

19. A compound of the following general formula:



wherein:

A represents an optionally substituted 5- or 6-membered heteroaryl ring

X is O, S or a group of Formula NR^{13} or $CR^{14}R^{15}$;

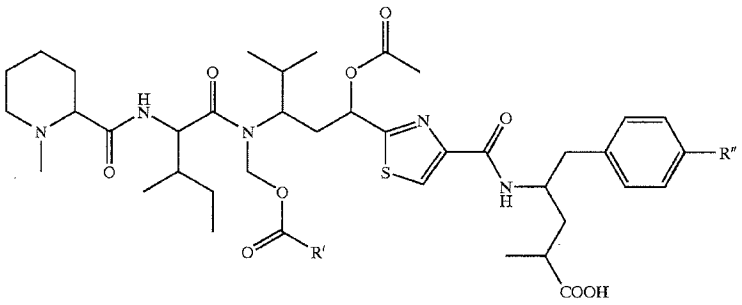
Y is O, S or a group of Formula NR^{16} and

R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16} are independently of each other H, alkyl, alkenyl, alkynyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, heterocycloalkyl, aralkyl or heteraaralkyl,

or two of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15} and R^{16} constitute part of a cycloalkyl or heterocycloalkyl;

or a pharmacologically acceptable salt, solvate, hydrate or a pharmacologically acceptable formulation thereof.

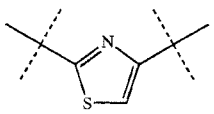
wherein compounds of Formula (1) are excluded,



(I)

wherein R' is H, alkyl, alkenyl, aryl, or heteroaryl, and R" is H, OH, alkyl, aryl or heteroaryl.

20. A compound of claim 19, wherein A has the following structure:



21. A compound of claim 19 wherein X is a CH_2 group.

23. A compound of claim 19 wherein Y is O.

24. A compound of claim 19 wherein R^1 is C_1-C_4 alkyl.

25. A compound of claim 19 wherein R^1 and R^3 together constitute a group of Formula $(CH_2)n$ wherein n is 2, 3, 4 or 5.

26. A compound of claim 19 wherein R^4 is H or methyl.

27. A compound of claim 19 wherein R^5 is H.

28. A compound of claim 19 wherein R^6 is C_1-C_6 alkyl, C_3-C_6 cycloalkyl or C_4-C_7 alkylcycloalkyl.

29. A compound of claim 19 wherein R^7 is H or methyl.

30. A compound of claim 19 wherein R^8 is a group of Formula CH_2OCOR^{17}, wherein R^{17} is C_1-C_7 alkyl or C_1-C_6 alkenyl.

31. A compound of claim 19 wherein R^9 is C_1-C_6 alkyl.

32. A compound of claim 19 wherein R^{10} is H or methyl.

33. A compound of claim 19 wherein R^{11} is H or a group of Formula (C═O)—(C_{1-4})alkyl.

34. A compound of claim 19 wherein R^{12} is a group of Formula $NR^{18}R^{19}$, wherein R^{18} is H or methyl and R^{19} is aralkyl or heteroaralkyl.

35. A pharmaceutical composition comprising a compound of claim 19 and optionally one or more carriers and/or adjuvants.

36. A method for treating a patient suffering from or susceptible to a tumor, immune disease, autoimmune disease, inflammatory disease or rheumatoid arthritis, comprising administering to the patient one or more compounds of claim 19.

37. The method of claim 36 wherein the patient is identified as suffering from a tumor, immune disease, autoimmune disease, inflammatory disease or rheumatoid arthritis, and the one or more compounds are administered to the identified patient.

38. A method for treating a patient suffering from cancer, comrpsing administering to the patient one or more compounds of claim 19.

39. The method of claim 38 wherein the patient is identified as suffering from cancer and the one or more compounds are administered to the identified patient.

* * * * *

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(71) **Anmelder** *(für alle Bestimmungsstaaten ausser US)*: GESELLSCHAFT FÜR BIOTECHNOLOGISCHE FORSCHUNG MBH (GBF) [DE/DE]; Mascheroder Weg 1, D–38124 Braunschweig (DE).

(72) **Erfinder; und**
(75) **Erfinder/Anmelder** *(nur für US)*: REICHENBACH, Hans [DE/DE]; Mascheroder Weg 1, D–38124 Braunschweig (DE). HÖFLE, Gerhard [DE/DE]; Mascheroder Weg 1, D–38124 Braunschweig (DE). SASSE, Florenz [DE/DE]; Mascheroder Weg 1, D–38124 Braunschweig (DE). STEINMETZ, Heinrich [DE/DE]; Mascheroder Weg 1, D–38124 Braunschweig (DE).

(74) **Anwälte:** BOETERS, Hans, D. usw.; Boeters & Bauer, Bereiteranger 15, D–81541 München (DE).

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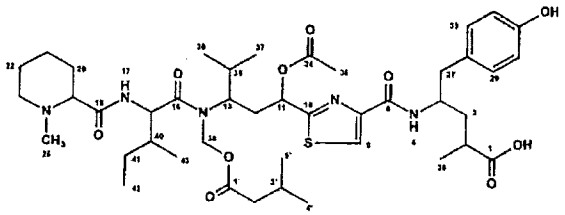
Veröffentlicht
Mit internationalem Recherchenbericht.
Vor Ablauf der für Änderungen der Ansprüche zugelassenen Frist. Veröffentlichung wird wiederholt falls Änderungen eintreffen.

(54) **Title:** COMPOUNDS WITH ANTIMYCOTIC AND CYTOSTATIC EFFECT, PREPARATION METHOD, AGENT CONTAINING THESE COMPOUNDS AND DSM 11 092

(54) **Bezeichnung:** VERBINDUNGEN MIT ANTIMYKOTISCHER UND CYTOSTATISCHER WIRKUNG, HERSTELLUNGSVERFAHREN, MITTEL UND DSM 11 092



Tubulysin A

(57) **Abstract**

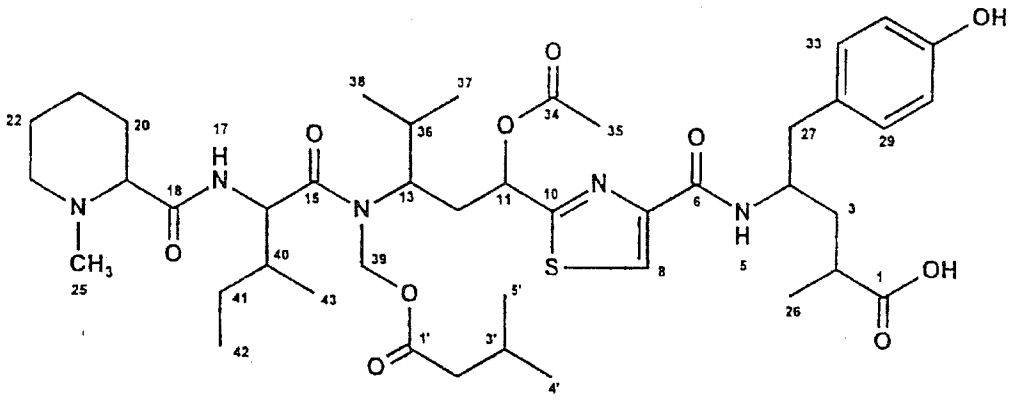
The invention relates to chemical compounds having antimycotic and cytostatic effect, a method for their preparation from *archangium gephyra* strain DSM 11 092, agent containing these compounds and said strain.

(57) **Zusammenfassung**

Die Erfindung betrifft chemische Verbindungen mit antimykotischer und cytostatischer Wirkung, ein Verfahren zu ihrer Gewinnung aus dem *Archangium gephyra*–Stamm DSM 11 092, Mittel mit den Verbindungen und dem Stamm.

Verbindungen mit antimykotischer und cytostatischer Wirkung, Herstellungsverfahren, Mittel und DSM 11 092

Gemäß einer ersten Ausführungsform betrifft die Erfindung eine chemische Verbindung der Formel



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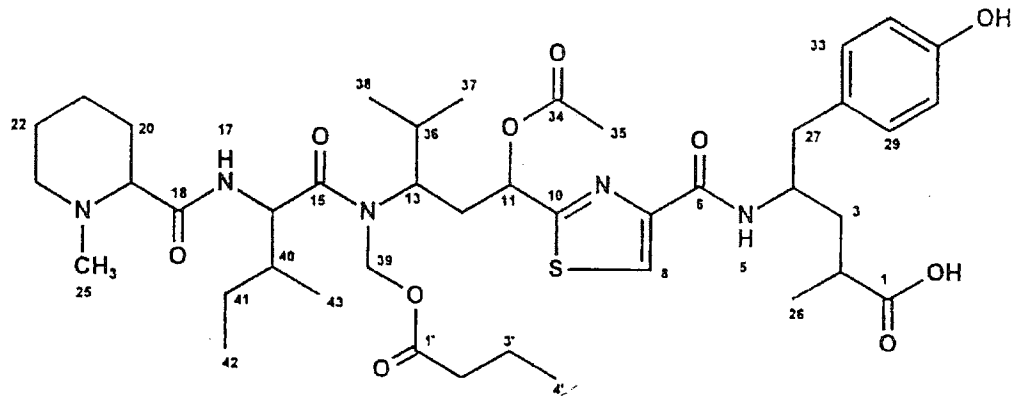
EE	Estland	**LR**	Liberia	**SG**	Singapur		

Gemäß einer weiteren Ausführungsform betrifft die Erfindung eine chemische Verbindung der Formel



Gemäß einer weiteren Ausführungsform betrifft die Erfindung eine chemische Verbindung der Summenformel $C_{43}H_{65}N_5O_{10}S$ und mit den folgenden Parametern:
^{1}H-NMR-Spektrum gemäß Tabelle 1 (Tubulysin A);
^{13}C-NMR-Spektrum gemäß Tabelle 1 (Tubulysin A);
UV-Spektrum (Methanol) $lambda_{max}$ (log epsilon): 225 (4,20), 250 (3,86) und 280 (3,20);
IR-Spektrum (KBr) ny: 3390, 2959, 2934, 2876, 1747, 1667, 1553, 1515 und 1233 cm^{-1}.

Gemäß einer weiteren Ausführungsform betrifft die Erfindung eine chemische Verbindung der Summenformel $C_{42}H_{63}N_5O_{10}S$ und mit den folgenden Parametern
^{1}H-NMR-Spektrum gemäß Tabelle 1 (Tubulysin B);
^{13}C-NMR-Spektrum gemäß Tabelle 1 (Tubulysin B);
UV-Spektrum (Methanol) $lambda_{max}$ (log epsilon): 225 (4,23), 250 (3,91) und 280 (3,26);
IR-Spektrum (KBr) ny: 3421, 2964, 2935, 2878, 1742, 1667, 1550, 1517 und 1235 cm^{-1}.

Gemäß einer weiteren Ausführungsform betrifft die Erfindung eine chemische Verbindung der Summenformel $C_{41}H_{61}N_5O_{10}S$ und mit einem R_t-Wert (HPLC) unter folgenden Bedingungen:

Säule: Nucleosil 100 C-18, 7 µm, 125 x 4 mm;
Laufmittel: Methanol/Wasser = 70/30 + 2 mM Ammoniumacetat (pH 5,0) + 10 mM Natriumdodecylsulfat;
Fluß: 1 ml/min;
Detektion: Diodenarray.

Gemäß einer weiteren Ausführungsform betrifft die Erfindung eine chemische Verbindungen mit antimykotischer und cytotoxischer Wirkung, dadurch gewinnbar, daß man
(a) *Archangium gephyra* DSM 11 092 in einem wässrigen Kulturmedium mit einem Gehalt an Kohlenstoff-Quellen, Stickstoff-Quellen, Schwefel-Quellen, Cyanocobalamin und Mineralsalzen aerob in Gegenwart eines Adsorberharzes kultiviert und
(b) das Adsorberharz vom Kulturmedium abtrennt und mit Methanol eluiert und vom Eluat das Methanol abzieht und
(c) die zurückbleibende Wasserphase mit Ethylacetat extrahiert, den Extrakt einengt und einen Rohextrakt gewinnt und
(d) den Rohextrakt einer Gelchromatographie mit Methanol als Laufmittel unterwirft und ein oder mehrere Fraktionen mit einem Gehalt an Verbindungen mit antimykotischer und cytostatischer Wirkung im UV bei 226 nm detektiert, abtrennt und einengt,
(e) das gewonnene Konzentrat an einer Umkehrphase mit Methanol/Ammoniumacetat-Puffer chromatographiert und durch Detektion im UV bei 226 nm
(e1) eine Fraktion mit einer rascher laufenden Verbindung sowie, zeitlich getrennt,
(e2) eine Fraktion mit einer langsamer laufenden Verbindung sowie, zeitlich getrennt,
(e3) eine Fraktion mit einer noch langsamer laufenden Verbindung abtrennt,
(f) von der gemäß (e1) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt,

(g) von der gemäß (e2) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt und
(h) von der gemäß (e3) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt.

Diese Verbindungen können dadurch gewinnbar sein, daß man bei Stufe (e) an einer C_{18}-Umkehrphase chromatographiert.

Gemäß einer weiteren Ausführungsform betrifft die Erfindung ein Verfahren zur Gewinnung von chemischen Verbindungen mit antimykotischer und cytostatischer Wirkung, dadurch gekennzeichnet, daß man
(a) *Archangium gephyra* DSM 11 092 in einem wässrigen Kulturmedium mit einem Gehalt an Kohlenstoff-Quellen, Stickstoff-Quellen, Schwefel-Quellen, Cyanocobalamin und Mineralsalzen aerob in Gegenwart eines Adsorberharzes kultiviert und
(b) das Adsorberharz vom Kulturmedium abtrennt und mit Methanol eluiert und vom Eluat das Methanol abzieht und
(c) die zurückbleibende Wasserphase mit Ethylacetat extrahiert, den Extrakt einengt und einen Rohextrakt gewinnt und
(d) den Rohextrakt einer Gelchromatographie mit Methanol als Laufmittel unterwirft und ein oder mehrere Fraktionen mit einem Gehalt an Verbindungen mit antimykotischer und cytostatischer Wirkung im UV bei 226 nm detektiert, abtrennt und einengt,
(e) das gewonnene Konzentrat an einer Umkehrphase mit Methanol Ammoniumacetat-Puffer chromatographiert und durch Detektion im UV bei 226 nm
(e1) eine Fraktion mit einer rascher laufenden Verbindung sowie, zeitlich getrennt,
(e2) eine Fraktion mit einer langsamer laufenden Verbindung sowie, zeitlich getrennt,
(e3) eine Fraktion mit einer noch langsamer laufenden Verbindung abtrennt,

(f) von der gemäß (e1) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt,
(g) von der gemäß (e2) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt und
(h) von der gemäß (e3) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt.

Gemäß einer weiteren Ausführungsform betrifft die Erfindung ein antimykotisches Mittel mit einem Gehalt an einer erfindungsgemäßen Verbindung.

Gemäß einer weiteren Ausführungsform betrifft die Erfindung ein cytostatisches Mittel mit einem Gehalt an einer erfindungsgemäßen Verbindung.

Schließlich betrifft eine Ausführungsform der Erfindung *Archangium gephyra* DSM 11 092.

Nachstehend wird die Erfindung durch experimentelle Angaben und 3 Figuren (Strukturformeln) näher erläutert.

A. Produktionsbedingungen

A.1. Produktionsstamm

Das Bakterium *Archangium gephyra* gehört zur Ordnung der Myxococcales (Myxobakterien), Unterordnung Cystobacterineae, Familie Archangiaceae. Der Produktionsstamm *Archangium gephyra* Ar 315 wurde im Februar 1973 von Dr. Reichenbach aus einer Probe von einem Komposthaufen im Botanischen Garten in Freiburg, Deutsch-

land, isoliert. Er wurde 1996 bei der Deutschen Sammlung von Mikroorganismen (DSM) unter der Nr. DSM 11 092 hinterlegt.

A.2. Stammkultur

Die Stammhaltung erfolgt auf Agarplatten, bevorzugt auf Hefe-Agar (VY/2-Agar). Dieses Medium enthält 0,5 % Bäckerhefe, 0,1 % $CaCl_2 \times 2H_2O$, 0,1 µg/l Cyanocobalamin und 1,2 % Agar. Der pH-Wert wird auf 7,4 eingestellt. Das Medium wird durch Autoklavieren sterilisiert. Die Plattenkulturen werden bei 30 °C bebrütet.

A.3. Morphologische Beschreibung

Die vegetativen Zellen sind lange, schlanke Stäbchen, etwa 6 bis 9 µm lang und 0,8 µm dick. Bedingt durch die Gleitbewegung der Bakterien, breiten sich die Kolonien rasch über die Kulturplatte aus. Die Schwarmkolonie auf Hefeagar ist dünn, filmartig, rötlich braun. Wie an dem um die Kolonien entstehenden Klärhof zu erkennen, werden die Hefezellen im Medium abgebaut. Auf diesem Medium bildet der Stamm oft blaßbräunliche Fruchtkörper, die aus mäandrierenden Wülsten aufgebaut sind und stark lichtbrechende Myxosporen enthalten. Letztere sind kurze, dicke, etwas unregelmäßige Stäbchen, etwa 2,5 bis 4 mm lang und 1,2 bis 1,8 mm dick.

A.4. Leistungen

Der Stamm Ar 315 produziert Substanzen, nämlich Tubulysine, die das Wachstum von Pilzen, humanen Krebszellen und anderen tierischen Zellkulturen hemmen. Die Hemmstoffe können sowohl aus den Zellen wie auch aus dem Kulturüberstand isoliert werden.

A.5. Produktion der Tubulysine

Die Substanzen werden während der logarithmischen bis hin zur stationären Wachstumsphase produziert. Eine typische Fermen-

tation verläuft wie folgt: Ein Fermentor mit 350 l Arbeitsvolumen wird mit 300 l Kulturmedium gefüllt (Zusammensetzung: 0,5 % Probion (Einzellerprotein der Fa. Hoechst); 1,0 % Stärke (Cerestar Krefeld); 0,2 % Glucose; 0,1 % Hefeextrakt; 0,1 % $MgSO_4 \times 7H_2O$; 0,1 % $CaCl_2 \times 2H_2O$; 0,1 µg/l Cyanocobalamin; Alternativen zu Probion sind Sojamehl oder Maiskleber). Der pH-Wert wird mit KOH auf 7,4 eingestellt. Zur Bindung der ins Medium freigesetzten Hemmstoffe wird dem Medium 1 % (V/V) eines Adsorberharzes (Amberlite XAD-16, Rohm & Haas) zugesetzt. Beimpft wird mit 10 l einer 3 Tage alten Vorkultur, die im gleichen Medium in einem entsprechend kleineren Fermentor erzeugt wurde. Fermentiert wird bei 30 °C mit einer Rührgeschwindigkeit von 150 U/min und einer Belüftungsrate von 10 Vol.-% pro min. Anfängliche Schaumbildung wird durch Zugabe von 50 ml Silikon-Antischaum (z. B. Tegosipon, Goldschmidt AG, Essen) verhindert. Der pH-Wert steigt im Laufe der Fermentation an. Der Anstieg wird durch Zugabe von 5-proz. Schwefelsäure auf 7,8 begrenzt. Die Fermentation wird nach 5 Tagen beendet.

B. Isolierung von Tubulysin A, B und C

Das Adsorberharz wird in einem Prozeßfilter (0,7 m^2, 100 Maschen (mesh)) von der Kultur abgetrennt, und mit 15 l Methanol im Verlauf von 3 h eluiert. Die Konzentration des Eluates erfolgt unter Vakuum bis zum Auftreten der Wasserphase, die anschließend dreimal mit Ethylacetat extrahiert wird. Nach Einengen der organischen Phase im Vakuum bei 30 °C Badtemperatur erhält man 36 g Rohextrakt.

Dieser Rohextrakt wird durch LH-20-Gelchromatographie (Säule: d = 20 cm, l = 100 cm, Fluß 45 ml/min, Detektion 226 nm) mit dem Laufmittel Methanol nach UV-Banden in 6 Fraktionen aufgetrennt, wobei Tubulysin A, B und C in der 2. Fraktion von 110 bis 130

min enthalten sind. Nach Einengen der betreffenden Fraktion trennt man in 3 Portionen auf einer Eurosil-Bioselect (100-20-C-18)-Säule (d = 4 cm, l = 48 cm) mit dem Laufmittel Methanol/0,05 M Ammoniumacetat-Puffer (pH 7,0) = 60/40 und einem Fluß von 8 ml/min. Die Detektion erfolgt bei 226 nm. R_t Tubulysin C 245 bis 260, Tubulysin B 260 bis 285 min, Tubulysin A 300 bis 320 min.

Nach Eindampfen der vereinigten Tubulysin A, Tubulysin B und Tubulysin C enthaltenen Fraktionen bis zur Wasserphase extrahiert man mit Ethylacetat und erhält nach dem Eindampfen im Vakuum und Trocknen 420 mg Tubulysin A, 240 mg Tubulysin B und 20 mg Tubulysin C.

Tubulysin A

$C_{43}H_{65}N_5O_{10}S$ [843]

DCI-MS (positiv-Ionen): 844.4543 für $[M+H]^+$

^1H- und ^{13}C-NMR siehe Tabellen 1 und 2

UV (Methanol) $lambda_{max}$ (log epsilon) = 225 (4.20); 250 (3.86); 280 (3.30)

IR KBr: ny = 3390; 2959; 2934; 2876; 1747; 1667; 1553; 1515; 1233 cm^{-1}

DC: R_f = 0.27
DC-Alufolie 60 F_{254} Merck. Laufmittel: Dichlormethan/Methanol = 9:1
Detektion: UV-Löschung bei 254 nm

HPLC: R_t = 9.7 min
Säule: Nucleosil 100 C-18 7 µm, 125 x 4 mm
Laufmittel: Methanol/Wasser = 70/30 + 2mM Ammoniumacetat (pH 5.0) + 10 mM Natrium-dodecylsulfat

Fluß: 1 ml/min
Detektion: Diodenarray

Tubulysin B

$C_{42}H_{63}N_5O_{10}S$ [829]

DCI-MS (positiv-Ionen): 830.4361 für $[M+H]^+$

^1H- und ^{13}C-NMR siehe Tabellen 1 und 2

UV (Methanol) $lambda_{max}$ (log epsilon) = 225 (4.23); 250 (3.91); 280 (3.26)

IR KBr: ny = 3421; 2964; 2935; 2878; 1742; 1667; 1550; 1517; 1235 cm^{-1}

DC: R_f = 0.25
DC-Alufolie 60 F_{254} Merck. Laufmittel: Dichlormethan/Methanol = 9:1
Detektion: UV-Löschung bei 254 nm

HPLC: R_t = 7.3 min
Säule: Nucleosil 100 C-18 7 µm, 125 x 4 mm
Laufmittel: Methanol/Wasser = 70/30 + 2 mM Ammoniumacetat (pH 5.0) + 10 mM Natrium-dodecylsulfat

Fluß: 1 ml/min
Detektion: Diodenarray

Tubulysin C

$C_{41}H_{61}N_5O_{10}S$ [815]

ESI-MS (positiv-Ionen): 816.6 für [M+H]

HPLC: R_t = 6.8 min
Säule: Nucleosil 100 C-18 7 µm, 125 x 4 mm.
Laufmittel: Methanol/Wasser = 70/30 + 2 mM Ammoniumacetat (pH 5,0) + 10 mM Natrium-dodecylsulfat

Fluß: 1 ml/min
Detektion: Diodenarray

Tabelle 1 ^{1}H-NMR data of tubulysines in [D_6] DMSO (600 MHz)

H	Tubulysin A			Tubulysin B		
	δ_H	m	J[Hz]	δ_H	m	J[Hz]
2-H	2.37	m		2.39	m	
3-H_a	1.57	m		1.55	m	
3-H_b	1.83	m		1.82	m	
4-H	4.10	m		4.11	m	
5-H	7.88	d	7.5	7.76	d	9.0
8-H	8.18	s		8.17	s	
11-H	5.74	dd	11.3, 1.4	5.75	dd	11.2, 1.6
12-H_a	2.09	m		2.08	m	
12-H_b	2.36	m		2.36	m	
13-H	4.35	m		4.35	m	
16-H	4.40	dd	9.0, 8.8	4.42	dd	9.0, 8.8
17-H	7.92	d	8.8	7.88	d	8.6
19-H	2.46	dd	7.6	2.47	m	
20-H_a	1.42	m		1.42	m	
20-H_b	1.51	m		1.52	m	
21-H_a	1.15	dd	12.5	1.16	m	
21-H_b	1.62	m	12.6	1.62	m	
22-H_a	1.36	m		1.38	m	
22-H_b	1.53	m		1.53	m	
23-H_a	1.94	m		1.93	m	
23-H_b	2.82	dd	11.4	2.83	dd	11.3
25-H_3	2.04	s		2.05	s	
26-H_3	1.04	d	7.0	1.05	d	7.0
27-H_a	2.66	m		2.68	m	
27-H_b	2.73	m		2.71	m	
29-H	6.96	d	8.4	6.96	d	8.4
30-H	6.61	d	8.4	6.62	d	8.3

32-H	6.61	d	8.4	6.62	d	8.3
33-H	6.96	d	8.4	6.96	d	8.4
35-H_3	2.10	s		2.11	s	
36-H	1.82	m		1.84	m	
37-H_3	0.67	d	6.5	0.68	d	6.6
38-H_3	0.97	d	6.5	0.97	d	6.4
39-H_a	5.26	d	12.0	5.27	d	12.0
39-H_b	6.19	d	12.0	6.20	d	12.0
40-H	1.93	m		1.95	m	
41-H_a	1.08	m		1.10	m	
41-H_b	1.49	m		1.49	m	
42-H_3	0.81	t	7.5	0.80	t	7.4
43-H_3	0.81	d	7.1	0.80	d	7.0
2'-H_a	2.13	m		2.15	m	
2'-H_b	2.15	m		2.18	m	
3'-H_a 3'-H_b	1.92 -	m		1.48 1.50	m m	
4'-H_3	0.82	d	6.9	0.82	t	7.0
5'-H_3	o.81	d	6.8			

Tabelle 2 ^{13}C-NMR data of tubulysines in [D_6] DMSO (600 MHz)

C	Tubulysin A δ_C	m	Tubulysin B δ_C	m
1	177.1	s	177.0	s
2	36.2	d	36.0	d
3	37.6	t	37.6	t
4	49.0	d	48.9	d
6	159.7	s	159.7	s
7	149.8	s	149.7	s
8	124.2	d	124.1	s
10	168.5	s	168.7	s
11	68.8	d	69.0	d
12	34.3	t	34.4	t
13	55.8 *	d	55.6 *	d
15	174.2	s	174.2	s
16	52.6	d	52.6	d
18	172.8	s	172.8	s
19	68.1	d	68.0	d
20	24.8	t	24.8	t
21	22.8	t	22.7	t
22	29.6	t	29.5	t
23	54.7	t	54.6	t
25	43.8	q	43.7	q
26	18.0	q	17.9	q
27	39.5	t	39.4	t
28	128.5	s	128.4	s
29	129.9	d	129.9	d
30	114.9	d	114.9	d
31	155.5	s	155.5	s
32	114.9	d	114.9	d

33	129.9	d	129.9	d
34	169.8	s	169.7	s
35	20.5	q	20.4	q
36	30.0	d	30.0	d
37	19.3	q	19.3	q
38	20.2	q	20.2	q
39	68.9 *	t	68.9 *	t
40	35.1	d	35.1	d
41	24.1	t	24.0	t
42	10.0	q	10.0	q
43	15.3	q	15.3	q
1'	171.3	s	171.8	s
2'	42.7	t	35.5	t
3'	25.0	d	17.6	t
4'	22.0	q	10.7	q
5'	22.0	q		

*δ_C gemessen bei 80° C

C. Wirkung

Die Tubulysine haben eine cytostatische Wirkung auf Pilze, humane Krebszellinien und andere tierische Zellkulturen (vgl. Tabelle). Sie führen in den Zellen zu einem raschen Abbau des Mikrotubuli-Gerüsts. Das Aktinskelett bleibt erhalten. Adhärent wachsende L929-Maus-Zellen vergrößern unter dem Einfluß der Tubulysine ihr Zellvolumen, ohne sich zu teilen, und entwickeln große Zellkerne, die dann in einem apoptotischen Vorgang zerfallen.

Wirkungsspektrum

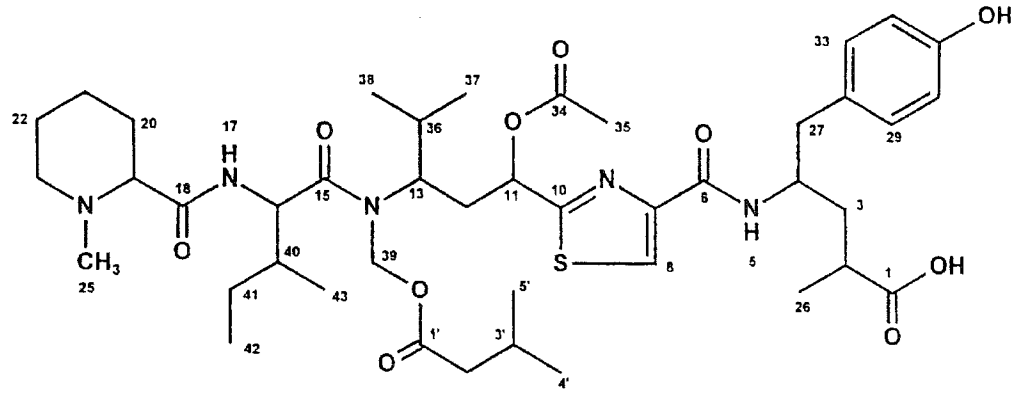
Pilze	Hemmhof [mm]	
	Tubulysin A	Tubulysin B
Aspergillus niger	20	18
Botrytis cinerea	23	18
Coprinus cinereus	20	
Pythium debaryanum	20	

Agardiffusionstest: 20 µg pro Testblättchen von 6 mm Durchmesser

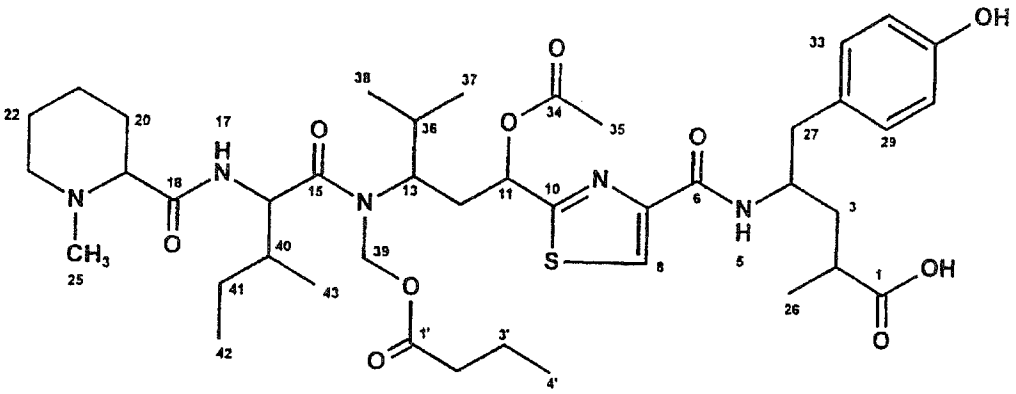
Humane Krebszellinien	IC_{50} [ng/ml]		
	Tubulysin A	Tubulysin B	Tubulysin C
KB-3-1 (DSM ACC 158)	0,01	0,02	0,1
K-562 (ATCC CCL 243)	0,1	0,2	1,5
HL-60 (ATCC CCL 240)	0,04	0,08	0,4
Tierische Zellinien			
L929, Maus (ATCC CCL 1)	0,2	0,4	2
Pt K2, *Potorous tridactylis* (ATCC CCL 56)	0,2	0,2	2

Patentansprüche

1. Chemische Verbindung der Formel



2. Chemische Verbindung der Formel



3. Chemische Verbindung der Summenformel $C_{43}H_{65}N_5O_{10}S$ und mit den folgenden Parametern:
^{1}H-NMR-Spektrum gemäß Tabelle 1 (Tubulysin A);
^{13}C-NMR-Spektrum gemäß Tabelle 1 (Tubulysin A);
UV-Spektrum (Methanol) $lambda_{max}$ (log epsilon): 225 (4,20), 250 (3,86) und 280 (3,20);
IR-Spektrum (KBr) ny: 3390, 2959, 2934, 2876, 1747, 1667, 1553, 1515 und 1233 cm^{-1}.

4. Chemische Verbindung der Summenformel $C_{42}H_{63}N_5O_{10}S$ und mit den folgenden Parametern
^{1}H-NMR-Spektrum gemäß Tabelle 1 (Tubulysin B);
^{13}C-NMR-Spektrum gemäß Tabelle 1 (Tubulysin B);
UV-Spektrum (Methanol) $lambda_{max}$ (log epsilon): 225 (4,23), 250 (3,91) und 280 (3,26);
IR-Spektrum (KBr) ny: 3421, 2964, 2935, 2878, 1742, 1667, 1550, 1517 und 1235 cm^{-1}.

5. Chemische Verbindung der Summenformel $C_{41}H_{61}N_5O_{10}S$ und mit einem R_t-Wert (HPLC) unter folgenden Bedingungen:
Säule: Nucleosil 100 C-18, 7 µm, 125 x 4 mm;
Laufmittel: Methanol/Wasser = 70/30 + 2 mM Ammoniumacetat (pH 5,0) + 10 mM Natriumdodecylsulfat;
Fluß: 1 ml/min;
Detektion: Diodenarray.

6. Chemische Verbindungen mit antimykotischer und cytotoxischer Wirkung, dadurch gewinnbar, daß man
(a) *Archangium gephyra* DSM 11 092 in einem wässrigen Kulturmedium mit einem Gehalt an Kohlenstoff-Quellen, Stickstoff-Quellen, Schwefel-Quellen, Cyanocobalamin und Mineralsalzen aerob in Gegenwart eines Adsorberharzes kultiviert und
(b) das Adsorberharz vom Kulturmedium abtrennt und mit Methanol eluiert und vom Eluat das Methanol abzieht und
(c) die zurückbleibende Wasserphase mit Ethylacetat extrahiert, den Extrakt einengt und einen Rohextrakt gewinnt und
(d) den Rohextrakt einer Gelchromatographie mit Methanol als Laufmittel unterwirft und ein oder mehrere Fraktionen mit einem Gehalt an Verbindungen mit antimykotischer und cytostatischer Wirkung im UV bei 226 nm detektiert, abtrennt und einengt,
(e) das gewonnene Konzentrat an einer Umkehrphase mit Methanol/Ammoniumacetat-Puffer chromatographiert und durch Detektion im UV bei 226 nm
(e1) eine Fraktion mit einer rascher laufenden Verbindung sowie, zeitlich getrennt,
(e2) eine Fraktion mit einer langsamer laufenden Verbindung sowie, zeitlich getrennt,
(e3) eine Fraktion mit einer noch langsamer laufenden Verbindung abtrennt,
(f) von der gemäß (e1) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt,
(g) von der gemäß (e2) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt und
(h) von der gemäß (e3) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt.

7. Chemische Verbindungen nach Anspruch 5, dadurch gewinnbar, daß man bei Stufe (e) an einer C_{18}-Umkehrphase chromatographiert.

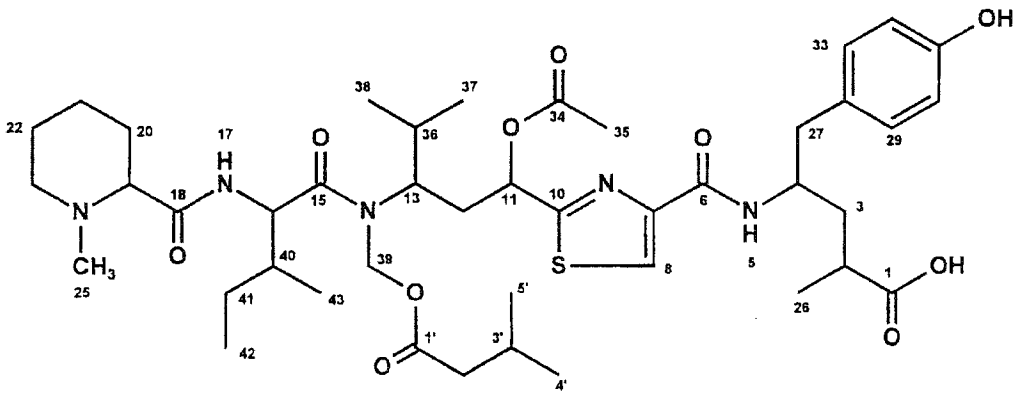
8. Verfahren zur Gewinnung von chemischen Verbindungen mit antimykotischer und cytostatischer Wirkung, dadurch **gekennzeichnet**, daß man
(a) *Archangium gephyra* DSM 11 092 in einem wässrigen Kulturmedium mit einem Gehalt an Kohlenstoff-Quellen, Stickstoff-Quellen, Schwefel-Quellen, Cyanocobalamin und Mineralsalzen aerob in Gegenwart eines Adsorberharzes kultiviert und
(b) das Adsorberharz vom Kulturmedium abtrennt und mit Methanol eluiert und vom Eluat das Methanol abzieht und
(c) die zurückbleibende Wasserphase mit Ethylacetat extrahiert, den Extrakt einengt und einen Rohextrakt gewinnt und
(d) den Rohextrakt einer Gelchromatographie mit Methanol als Laufmittel unterwirft und ein oder mehrere Fraktionen mit einem Gehalt an Verbindungen mit antimykotischer und cytostatischer Wirkung im UV bei 226 nm detektiert, abtrennt und einengt,
(e) das gewonnene Konzentrat an einer Umkehrphase mit Methanol Ammoniumacetat-Puffer chromatographiert und durch Detektion im UV bei 226 nm
(e1) eine Fraktion mit einer rascher laufenden Verbindung sowie, zeitlich getrennt,
(e2) eine Fraktion mit einer langsamer laufenden Verbindung sowie, zeitlich getrennt,
(e3) eine Fraktion mit einer noch langsamer laufenden Verbindung abtrennt,
(f) von der gemäß (e1) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt,
(g) von der gemäß (e2) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt und

(h) von der gemäß (e3) gewonnenen Fraktion das Methanol abzieht, die zurückbleibende Wasserphase mit Ethylacetat extrahiert, eindampft und trocknet und die Verbindung gewinnt.

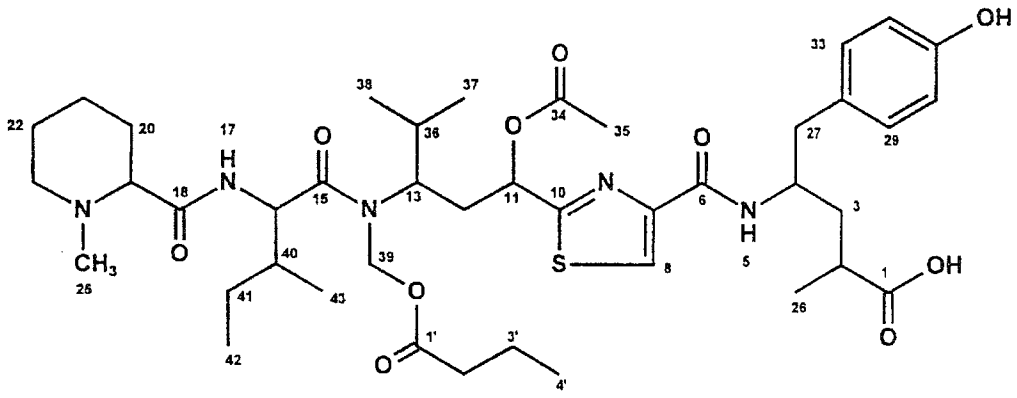
9. Antimykotisches Mittel mit einem Gehalt an einer Verbindung gemäß einem der Ansprüche 1 bis 7.

10. Cytostatisches Mittel mit einem Gehalt an einer Verbindung gemäß einem der Ansprüche 1 bis 7.

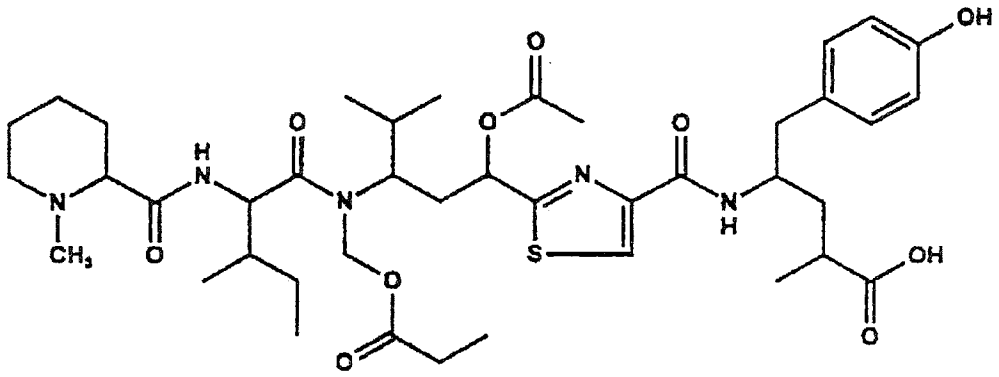
11. *Archangium gephyra* DSM 11 092.



Tubulysin A



Tubulysin B



Tubulysin C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/05095

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/078 C12P1/04 C12R1/01 A61K38/05 C12N1/20
//(C12P1/04,C12R1:01)

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 13094 A (BIOTECHNOLOG FORSCHUNG GMBH) 8 July 1993	
A	F. SASSE ET AL: "Gephyronic acid, a novel inhibitor of Eukariotic protein synthesis from Archangium gephyra" THE JOURNAL OF ANTIBIOTICS, vol. 48, no. 1, 1995, pages 21-25, XP002051795	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

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"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 1998	26/01/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Cervigni, S

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 97/05095

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9313094 A	08-07-93	DE 4142951 C	13-05-93
		AU 3257793 A	28-07-93

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
PCT/EP 97/05095

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 6 C07K5/078 C12P1/04 C12R1/01 A61K38/05 C12N1/20
//(C12P1/04,C12R1:01)

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
IPK 6 C07K C12P C12N

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie°	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
A	WO 93 13094 A (BIOTECHNOLOG FORSCHUNG GMBH) 8.Juli 1993 ---	
A	F. SASSE ET AL: "Gephyronic acid, a novel inhibitor of Eukariotic protein synthesis from Archangium gephyra" THE JOURNAL OF ANTIBIOTICS, Bd. 48, Nr. 1, 1995, Seiten 21-25, XP002051795 -----	

☐ Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

☒ Siehe Anhang Patentfamilie

° Besondere Kategorien von angegebenen Veröffentlichungen :
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"&" Veröffentlichung, die Mitglied derselben Patentfamilie ist

2

Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
13.Januar 1998	26/01/1998

Name und Postanschrift der Internationalen Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cervigni, S

Formblatt PCT/ISA/210 (Blatt 2) (Juli 1992)

INTERNATIONALER RECHERCHENBERICHT

Angaben zu Veröffentlichungen, die zur selben Patentfamilie gehören

Internationales Aktenzeichen
PCT/EP 97/05095

Im Recherchenbericht angeführtes Patentdokument	Datum der Veröffentlichung	Mitglied(er) der Patentfamilie	Datum der Veröffentlichung
WO 9313094 A	08-07-93	DE 4142951 C	13-05-93
		AU 3257793 A	28-07-93